



**School of Land and Food**

**Evaluation of non-aerated compost teas for  
suppression of potato diseases**

By

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(Pharmacognosy, Msc)

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## **Declarations**

This is to certify that:

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution and to the best of my knowledge contains no material previously published or written by any other person, except where duly acknowledged in the thesis.

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March 2017

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## Abstract

The use of water extracts from composted organic waste to suppress crop diseases has received attention in some parts of the world over recent decades. These extracts, also known as compost tea, are produced from compost that is fermented in water (either with or without aeration) for a specific period, filtered, and applied as a foliar spray or soil drench to control plant diseases. Compost tea incorporates soluble organic matter, beneficial microorganisms and macro and micronutrients, thus it is claimed that application to soil-plant systems improves soil biology and fertility for subsequent plant growth. Non-aerated compost tea (NCTs) production is a low-cost method to prepare an anaerobic tea from compost and previous studies have shown that NCTs are effective in limiting a wide range of foliar and soil borne disease in most of the pathosystems evaluated. However, how production factors (e.g. compost type, timing of application, concentration, and addition of adjuvants) influence effectiveness of NCTs has not been fully explored.

The growing awareness of negative consequences on human and environmental health associated with reliance on synthetic pesticides has led to increased interest in alternative crop management solutions. In developing countries, such as Ethiopia, alternatives that are low-cost are particularly worthy of further development. Therefore, there is a compelling reason for researching NCTs as disease suppressants by designing studies to better understand the involved factors and mechanisms and inform practical use. The research presented in this thesis was designed to evaluate suppressive efficacy of different types of NCTs against economically important pathogens of potato (*Solanum tuberosum* L.), an important food source in Ethiopia, in different experimental settings.

Initially, *in vitro* bioassays of NCTs from two Tasmanian compost sources (a commercial product and a vineyard compost) were tested against fungal pathogens of potato: *Alternaria solani* and *Rhizoctonia solani*. Firstly, it was demonstrated that the biotic component of the two NCTs was required to inhibit growth of the pathogen mycelium, while NCTs sterilized by filtration led to no suppression of pathogen growth. Secondly, it was found that maximum inhibition differed with NCT type and pathogen, for example, application of the commercial compost tea resulted in up to 74% inhibition of *A. solani* mycelium, 85% of *R. solani* (isolate 422) but only 36% for *R. solani* (isolate 299). An assay with detached potato leaves tested

the efficacy of the NCT derived from the commercial compost, with or without an additive carbohydrate gum, against brown leaf spot symptom caused by *Alternaria alternata* and found that while application of the compost tea significantly reduced disease severity, there was no measured difference when gum was added. Important physico-chemical characters of the NCTs used in the above experiments, and their respective parent compost were described. In addition, the bacterial and fungal communities were analysed by high throughput next-generation sequencing of the 16S rRNA and ITS genes and found to contain higher numbers of bacterial operational taxonomic units than fungal. The microbial community structure of the NCTs and chemical characters extracted from the parent composts are important factors affecting the suppressive abilities of the NCTs.

Studies with pot-grown potato plants were carried out in Ethiopia with NCTs made from different compost sources and using the economically important bacterial wilt pathogen *Ralstonia solanacearum* (biovar II). Three different kinds of mature and cured compost sources typically present in Ethiopia (agricultural waste compost, solid municipal waste compost and vermicompost) were used to prepare NCTs. Compost source and timing of application were the two most important factors that influenced reduction of bacterial wilt symptoms. In this experiment, NCTs made from agricultural waste compost were more effective to reduce disease severity than those made from solid municipal waste or vermicompost tea. Moreover, application of NCT at the same time that potato tubers were planted and inoculated with pathogenic *R. solanacearum* led to greater disease reduction than when applied 7 days before or after introducing the pathogen inoculum to the soil and planting the tubers. The most effective treatment resulted in a 2.5-fold reduction in disease compared to the non-treated controls, based on the “area under the disease progress curve” parameter.

The effectiveness in suppression of bacterial wilt development of these same NCTs when combined with tree derived gum sources as adjuvants was further investigated in pot-trials in Ethiopia where compost tea was applied as a drench to planted tubers and then a spray as plants emerged. In this case, both the NCT type and type of the added gum (gum myrrh or opoponax) were found to be important factors for enhancing bacterial wilt suppression. As for the previous study, the NCT made from agricultural waste led to greater disease suppression than the other NCT types, and addition of myrrh gum led to the greatest

reduction (over 2-fold) of disease severity. Therefore, the addition of gum was shown to be beneficial.

Given the agricultural waste NCTs were generally most effective in controlling several key pathogens of potato, either *in-vitro* or *in-vivo*, an on-farm composting trial was carried out using the readily available agricultural waste materials pertinent to small scale farming practices in the central high land area of Ethiopia. Factors examined included different plant substrates (maize, wheat and cowpea straw and grasses, or coffee husk) and compost ages. The pit-composting method used led to long mesophilic phases (which did not exceed 45°C) as opposed to aerobic and open windrow compost methods that can reach up to 70°C in the thermophilic stage. Physico-chemical parameters of both the parent compost and the NCTs were measured for samples collected at three different stages of composting and values of pH, EC, exchangeable cations and nutrients, and the C: N ratio were found to be in the optimum range when compared with literature reports of agricultural waste compost materials. The shifts in microbial communities of NCTs over the stages of composting were thoroughly studied based on next-generation sequencing of 16S and ITS genes for bacterial and fungal communities, respectively. Bacterial phyla such as *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and *Chloroflexi* were identified with marked shifts in relative abundances over stages of composting. Similarly, fungal taxa, mainly *Ascomycota*, *Basidiomycota*, *Cryptomycota*, *Entomophthoromycota* and *Glomeromycota*, were abundant phyla accompanied with variation in relative abundances across the different stages of composting considered in the experiment.

In conclusion, this thesis demonstrates that non-aerated compost teas that are prepared from a mixture of on-farm waste materials can reduce both soil borne and foliar disease of potato, and that efficacy can be further enhanced when carbohydrate rich gum is added as an adjuvant. The potential of this low-cost resource to be made and used on-farm in field situations should be trialled to develop practical recommendations to enable integration in to crop protection strategies, particularly in developing countries.

## Co-authorship statement

The PhD thesis has four experimental chapters. Of these, one chapter is published, one under review, and the remaining chapters are intended for submission to respective journals.

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### **Chapter 3: Factors affecting efficacy of compost tea against selected fungal pathogens of potato**

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As primary supervisor I agree with the stated proportion of contribution to the chapters, which are published or submitted to journals or publishers.

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# Contents

Declarations .....	i
Acknowledgements .....	ii
Abstract.....	iii
Co-authorship statement.....	vi
Other publications and communications arising from this research .....	vii
Chapter 1 .....	1
INTRODUCTION.....	1
Objectives of the thesis .....	5
Chapter 2 .....	7
LITERATURE REVIEW: THE USE OF COMPOST TEA IN THE MANAGEMENT OF PLANT DISEASE .....	7
2.1. Introduction .....	7
2.2 Compost and disease suppression .....	7
2.3. Types of Compost teas.....	10
2.4. Factors affecting the quality and efficacy of NCTs .....	12
2.5. Mechanisms of disease suppression by NCT .....	16
2.6. The role of adjuvant addition to NCT .....	19
2.7. Summary .....	21
Chapter 3 .....	22
FACTORS AFFECTING EFFICACY OF COMPOST TEA AGAINST SELECTED FUNGAL PATHOGENS OF POTATO.....	22
3.1. Abstract.....	22
3.2. Introduction .....	23
3.3 Materials and Methods .....	27
<i>Compost sources and non-aerated compost tea .....</i>	<i>27</i>
<i>Microbial diversity of the NCTs .....</i>	<i>29</i>
<i>Fungal pathogens .....</i>	<i>30</i>
<i>In vitro mycelial growth inhibition .....</i>	<i>31</i>
<i>Detached leaf assays – experimental design and visual assessment .....</i>	<i>32</i>
<i>Detached leaf assays - observation with ESEM and SEM .....</i>	<i>34</i>
<i>Data analysis.....</i>	<i>35</i>

3.4. Results.....	35
<i>Physico-chemical properties of the NCTs.....</i>	35
<i>Microbial community structure and diversity.....</i>	36
<i>Effect of NCT on mycelial growth .....</i>	39
<i>Detached leaf assays – effect of NCT on infection by A. alternata.....</i>	43
<i>Detached leaf assay - visualisation of CCT microbe-pathogen interaction .....</i>	45
3.5. Discussion .....	50
Chapter 4 .....	57
DIVERSE MICROBIAL COMMUNITIES IN NON-AERATED COMPOST TEAS SUPPRESS BACTERIAL WILT	57
4.1. Abstract.....	57
4.2. Introduction .....	58
4.3. Materials and Methods .....	61
<i>Composting conditions and feedstock composition .....</i>	61
<i>Preparation of compost tea .....</i>	61
<i>Isolation and culture of Ralstonia solanacearum .....</i>	62
<i>Biochemical characteristics of Ralstonia solanacearum isolates .....</i>	63
<i>Characterisation of compost and NCTs .....</i>	63
<i>Statistical analysis.....</i>	65
<i>Greenhouse experiment.....</i>	65
4.4. Results.....	68
<i>Characteristics of Ralstonia solanacearum .....</i>	68
<i>Physicochemical characteristics of parent compost and compost teas .....</i>	69
<i>Effect of NCT on bacterial wilt disease .....</i>	70
<i>Microbial community structure and diversity.....</i>	72
4.4. Discussion .....	81
Chapter 5 .....	86
TREE-DERIVED GUM ENHANCES THE EFFECT OF NON-AERATED COMPOST TEA IN SUPPRESSING	
BACTERIAL WILT OF POTATO.....	86
5.1. Abstract.....	86
5.2. Introduction .....	87
5.3. Materials and Methods .....	89
<i>Bacterial isolate and inoculum preparation .....</i>	89
<i>Preparation and characterization of compost tea.....</i>	89
<i>Extraction of gum from tree exudates.....</i>	91

<i>Greenhouse experiment</i> .....	92
5.5. Results.....	94
<i>Characters of the gum sources (exudates)</i> .....	94
<i>Effect of treatments on bacterial wilt development</i> .....	95
5.6. Discussion .....	98
Chapter 6 .....	101
SUCCESSION OF MICROBIAL COMMUNITIES IN NON-AERATED COMPOST TEAS FROM AGRICULTURAL WASTES DURING UNDERGROUND COMPOSTING .....	101
6.1. Abstract .....	101
6.2. Introduction.....	102
6.3. Materials and Methods .....	105
<i>Composting system</i> .....	105
<i>Preparation of non-aerated compost tea</i> .....	107
<i>Physico-chemical measurements of compost and NCTs</i> .....	107
<i>Microbial diversity of the NCTs</i> .....	108
<i>Statistical analysis</i> .....	109
6.4. Results .....	110
<i>Physicochemical characteristics</i> .....	110
<i>Amplicon sequencing</i> .....	112
<i>Microbial diversity and community structure</i> .....	115
<i>Variation in community compositions</i> .....	118
<i>Community diversity</i> .....	123
<i>Physico-chemical characteristics of parent composts</i> .....	125
<i>Physico-chemical properties of the compost teas</i> .....	126
<i>Changes in microbial communities</i> .....	127
Chapter 7 .....	135
GENERAL DISCUSSION .....	135
References .....	140

# Chapter 1

## INTRODUCTION

Associated with exponential increases in the world population, global food security is increasingly at risk and particularly so in many developing countries. Reliable and adequate food crop production is critical in addressing this risk and gap in production (Godfray *et al.*, 2010; Sundström *et al.*, 2014). FAO (2009) estimated the world population to be 9.1 billion by 2050, which is an increase of 34 % compared to the current figure. It has also been estimated that agricultural production must increase by 70 % by 2050 from what is currently being produced annually.

There are however, many pressing challenges and threats posed to agricultural production at a global scale. Some factors are direct consequences of the boom in population on agricultural production and productivity which include a decline in arable land because of land degradation, urban expansion and conversion of crops and croplands for non-food production (Nellemann, 2009; Zhan *et al.*, 2014). Other factors, such as plant disease, are ongoing biotic challenge and continue to be a major contributing factor to food shortage worldwide (Oerke, 2006; Strange and Scott, 2005). Yield and quality of the main food crops: cereals (mainly rice, wheat, maize) and potatoes and cassava as important staple root food crops (Alexandratos and Bruinsma, 2012; Anderson *et al.*, 2004; Gibson and Kurilich, 2013), are consistently lost due to diseases caused by pathogens and damage from pests

worldwide. Major groups of pathogens such as viruses, bacteria, Oomycetes, fungi, nematodes, and also parasitic plants are known to significantly impact food crops both in the field and after harvest (Strange and Scott, 2005). Protecting crops from disease causing pathogens is therefore an essential task for provision of food in sufficient quantity and quality for the increasing world population.

A range of different types of crop disease control methods are practiced in modern agriculture. These include elimination or reduction of the pathogen inoculum and restriction of its spread, via use of synthetic or organic pesticides, biological control, developing resistant varieties through breeding programmes, and cultural methods such as removing diseased plants and sanitation of field and farm implements (Kahn, 1991; Oerke and Dehne, 2004; Strange and Scott, 2005; Zadoks, 2003). These different categories of disease management approaches are generally employed to prevent, mitigate or control economically important crop disease and pests (Maloy, 2005; Pal and Gardener, 2006).

Development of crop disease results from complex interactions among biotic and abiotic factors that include hosts, pathogen and environments. The success or failure of any type of crop protection strategy is dependent not only on the interaction of these factors but also on the crop production system in question, which in turn is influenced by the socio-economic conditions, and the availability and affordability of the strategy to the growers. In farming systems that operate at small scale, for example, the range of choices available for disease management is typically limited (Letourneau and van Bruggen, 2006; Wilson and Tisdell, 2001).

Potato (*Solanum tuberosum* L.) ranks fourth in terms of production and consumption as a staple food worldwide following the major cereal crops: rice, wheat, and maize (DeFauw *et*

*al.*, 2012; Muthoni and Nyamongo, 2009). It is becoming a more important foodstuff because of its good nutritional qualities and its growth as a staple food crop in more than 100 countries under various climatic conditions (FAO, 2008; Fiers *et al.*, 2012). Major abiotic constraints for production include temperature and rainfall, with extreme events (e.g. hail, drought) presenting risk of loss. Both extreme weather and diseases and pests were the major risks reported by growers in a recent South African survey (Van der Waals *et al.*, 2016). Potatoes are highly impacted by large number of both soil borne and foliar diseases. It has been documented that it suffers from more than 40 kinds of diseases and pests caused by fungi, bacteria, viral pathogens, nematodes and insects (Fiers *et al.*, 2012).

In most east African countries, yield loss due to late blight caused by the fungal pathogen *Phytophthora infestans* and bacterial wilt caused by *Ralstonia solanacearum*, are regarded as the top-ranked problems in potato production (Gildemacher *et al.*, 2009). The high incidence of these diseases is due to many factors that include lack of disease-free seed systems, inappropriate use of control chemicals, lack of proper sanitation, crop rotation, and lack of varietal resistance among other factors (Schulte-Geldermann, 2013). Similarly, fungal diseases such as early blight, fusarium wilt, black scurf, and others have been documented as serious diseases of potato that affect both the crop/foilage and the tubers (Arora and Khurana, 2004; Secor and Gudmestad, 1999; Irshad and Naz, 2014). It was noted that for many of these diseases, there is often little correlation between application of chemical controls and disease severity, as growers often do not apply them at times of high disease risk due to lack of awareness (Van der Waals *et al.*, 2016)

The threat of bacterial wilt to intensive potato production is expected to increase in the Eastern African highlands as these areas have high frequency of field reinfection due to

short rotation periods and limitation of reliable clean seed sources (Gildemacher *et al.*, 2009; Schulte-Geldermann, 2013; Hirpa *et al.*, 2010). The disease is difficult to control and there is no universal method available for potato and other solanaceous crops. Resistant cultivars and breeding lines often are not effective since this depends on specificity of strains and climatic conditions to which the plants are exposed. The use of soil fumigants or chemicals to control bacterial wilt is environmentally destructive, expensive, and largely ineffective in most cases (Champoiseau *et al.*, 2009; Muthoni *et al.*, 2012).

Approaches worthy to pursue for bacterial wilt control consist primarily of phytosanitation and other cultural practices, along with the effect of organic soil amendments and the evaluation of antagonistic agents (Schulte-Geldermann, 2013). Composted organic wastes in different forms, such as a bioorganic fertilizer (Wu *et al.*, 2014; Liu *et al.*, 2015), solid compost amendment (Schönfeld *et al.*, 2003), and in one case a fermented compost extract/compost teas (Islam *et al.*, 2014) have been shown to control bacterial wilt in different pathosystems. However, little research has been conducted in evaluating compost tea as a suppressant of bacterial wilt in potatoes. In contrast, the use of compost teas as fungal disease suppressant has been popular in certain organic and biodynamic systems over the last decade (Litterick *et al.*, 2004; Scheuerell and Mahaffee, 2006). Compost tea has been found to suppress soil borne and foliar fungal pathogens of tomato and potato (Koné *et al.*, 2010 ; On *et al.*, 2015a).

Despite mounting pressure to replace synthetic pesticide inputs with alternative crop management tools which include the use of compost teas, the generation of highly effective and reproducible solutions is an ongoing research challenge (Litterick *et al.*, 2004). This is because, the effectiveness of compost tea (and other biological control approaches) in

suppressing either the growth of the pathogen or the resultant disease is influenced by many factors. The choice to use aerated or non-aerated compost tea, the type and composition of substrates used for composting, age of compost, the biological and chemical characteristics of compost and the compost teas, the use of additives, the pathosystem in question, and frequency and timing of application, among other factors, have been reported to affect the efficacy of compost tea application in disease control (Evans and Percy, 2014; Siddiqui *et al.*, 2015; St Martin, 2015). Besides, because the basis of the method is on-farm production, there have been no or minimal commercial interests to fund the research required to optimise practical methods, unlike the support invested in development of commercial products by agricultural companies.

## Objectives of the thesis

The main aim of the studies presented in this thesis was to evaluate disease suppressive efficacies of non-aerated compost teas prepared from different compost sources against selected bacterial and fungal pathogens and their resultant protection of potato diseases, via experiments conducted in Tasmania and Ethiopia.

The specific objectives were to:

- Evaluate suppressive efficacies of NCTs made from mature compost sources against potato fungal pathogens: *Alternaria solani*, *Alternaria alternata*, and *Rhizoctonia solani* and define the mechanisms of suppression;



- Examine suitability of NCTs made from variable sources in suppressing bacterial wilt of potato caused by *Ralstonia solanacearum* and the effect of timing and mode of application;
- Evaluate the effect of carbohydrate gum addition to NCTs as an adjuvant in bacterial wilt disease suppression;
- To characterise physico-chemical and microbiological features of NCTs made from different stages of composting organic wastes.

# Chapter 2

## LITERATURE REVIEW: THE USE OF COMPOST TEA IN THE MANAGEMENT OF PLANT DISEASE

### 2.1. Introduction

This literature review provides an overview of the use of compost and compost teas in agricultural and horticultural systems and the potential benefits for disease suppression. It presents the background and most current knowledge on factors that influence disease suppression by compost teas and putative mechanisms of action, with a focus on horticultural crops and reference to smallholder farming applications.

### 2.2 Compost and disease suppression

Compost is a decomposition product of biodegradable organic materials. During the biological process, a consortium of microorganisms are involved in the degradation process, followed by a succession of microbes which re-colonize the final solid particulate product with metabolically active microbial populations (Boulter *et al.*, 2002). Compost has been applied for centuries to agricultural and horticultural fields for the purpose of crop nutrition and soil fertility improvement. Pioneer activities during the 1960's in the USA made a finding that amendments of potting mix with compost from tree bark in nurseries could suppress *Phytophthora* root rots (Hoitink *et al.*, 1997). This observation has led to a great deal of scientific effort in investigating the plant disease suppressive abilities of compost and numerous positive outcomes have been reported on different pathosystems as reviewed by

Litterick *et al.* (2004). The nature of the compost life cycle such as the carbon level, nitrogen level, the C: N ratio, maturation and cooling phases, as well as characteristic succession of microbial communities during composting cycle, have all been associated with establishment of compost disease suppressive abilities (Hadar and Papadopoulou, 2012). Compost-based disease suppression is regarded as a microbially mediated process because of the role microbial populations play, primarily in two ways: a) general suppression due to the involvement of the whole microbial community b) specific suppression due to the presence of specific microbial taxa taking part in reduction of the plant disease (Hadar, 2011; Hadar and Papadopoulou, 2012). In addition, an equally important aspect is the physico-chemical features of compost that play a significant role in suppression of disease (Hoitink and Changa, 2002). The success of soil borne disease management by compost is a wide spread phenomenon. However, the practicality of this application, particularly in field situations, is still limited because of a lack of consistency in production factors and quality of compost.

Application of compost in a liquid form to horticultural crops, in an aim to help prevent and/or control both foliar and soil borne plant diseases, has been a popular practice in organic farming for quite some time. Historically, the use of 'compost water' was an ancient practice in gardening mainly for soil fertility and root growth as indicated in a review by Scheuerell and Mahaffee (2002). Large sets of experimental evidence for the potential of these compost extracts as suppressants of wide range of plant disease, however, have only come to light since the early 1990's (McQuilken *et al.*, 1994; Weltzien, 1991; Yohalem *et al.*, 1996), and have continued to be an important topic in the research of alternative crop protection methods.

The water preparation of compost has been described interchangeably by different terminologies including compost leachate, steepage, compost slurry, compost tea, and water fermented compost extract among others by in the reports of different authors. However, in the last two decades, with the advent of increasing numbers of horticultural growers producing and using this compost product as a plant protectant, the name “compost tea” has become popular and is now a standard description among researchers and practitioners (Scheuerell and Mahaffee, 2002). Therefore, compost tea is a fermentation of solid compost in water in a specific ratio for a defined length of time and that is then applied to the plant and/or soil with an aim to suppress plant disease and aid healthy plant growth. There are two major types of compost tea, aerated compost tea (ACT) and non-aerated compost tea (NCT), primarily based on the presence or absence of oxygen during the fermentation process, respectively.

The use of compost tea as a disease suppressant is generally different from the reported use of the solid compost controlling soil borne diseases when applied in a container media. The compost teas are liquid and applied or sprayed by apparatus directly on the plant surfaces, and in most cases, they act directly, through different mechanisms, to suppress the growth and germination of the pathogen (Brinton and Droffner, 1995). Currently, large numbers of experimental trials have been conducted to test compost teas on a wide range of pathosystems and yet its full application in field settings as crop protectant is still limited as many factors influence its production and application.

In Ethiopia, farmers in the central high lands have a strong tradition of using composted materials of animal manure and green yard waste for soil amendments and fertility restoration (Negassa *et al.*, 2001; Teklu and Hailemariam, 2009). However, knowledge of

production and application of compost extracts for the control of crop disease is less known, and otherwise could be produced locally as a crop protectant given that these solutions are proven effective against economically important horticultural crop diseases. Moreover, the beneficial effects of both compost and compost teas in suppressing disease-causing pathogens are apparently more relevant in low-input and organic agriculture where the use of pesticides/chemicals are entirely prohibited and/or where there are limited financial resources to cover the ever increasing cost of synthetic pesticide inputs. Therefore, any potential for improvement in the effectiveness of compost tea as an alternative input to suppress economically important diseases of staple food crop, such as potato, will have a tremendous potential for adoption by organic and small to urban-scale horticultural farms.

The aim of this review is therefore, to highlight the existing knowledge on the research of compost teas and the important determining factors of their efficacy in disease management.

### 2.3. Types of Compost teas

Aerated compost tea (ACT) and non-aerated compost tea (NCT), are the two dominant methods of compost tea preparation, based on whether oxygen is introduced during the fermentation process or not, respectively (Brinton and Trankner, 1996; Scheuerell and Mahaffee, 2002). Several reviews have evaluated both types of compost teas in a wide range of horticultural and ornamental plants in various experimental settings, often resulting in variable magnitudes of disease suppression (Deepthi and Reddy, 2013; Evans and Percy, 2014). According to the comparative evaluation work by Scheuerell and

Mahaffee (2006), ACT made from compost, water, constant oxygen supply and nutrient additives showed greatly increased microbial populations over a 12-36 hour period. In contrast, NCT, which had no aeration, was produced for a period of two weeks to develop comparable microbial populations. In their work, there was no significant difference in the efficacies between NCT and ACT application on suppression of gray mould of geranium. The main factor influencing suppressive efficacy was the character of parent compost. For example, NCTs made from yard trimmings compost and chicken manure composts had highest disease suppression compared to other compost types used in the study. Aerating and supplementing the system with nutrient additives enhanced the level of culturable microbes, but that did not correlate with the level of disease suppression. However, NCT without nutrient additive and fermented for 14 days was consistently suppressive. Similarly, in a study to suppress bacterial spot disease on tomato by Al-Dahmani *et al.* (2003), there was no significant difference between the effect of ACT and NCT that were produced from a range of compost sources and nutrient additives.

Based on many previous scientific reports, the decision to use either type of compost tea has not merely been based on the disease suppressiveness of the type of tea. Rather, it has been mainly because aerated teas could be made in a relatively in short period with typical fermentation times of up to 36 h compared to the typical extraction time of up to two weeks required for the production of NCT. Brinton *et al.* (2004) stated that better disease control usually resulted from the longer brewed non-aerated teas and that the quality of starting compost and hygienic circumstances of tea production is a more important factor than aerating the process to make ACTs.

Once the NCT making process is completed, the filtered teas obtained from the compost-water ferment typically contain a large number of microorganisms, nutrients and organic compounds from compost mineralization (Wickland *et al.*, 2001). There are also claims that microbial secondary metabolites such as phenols and amino acids produced during fermentation aid induction of plant-defence responses and healthy growth (Koné *et al.*, 2010; Van Zwieten *et al.*, 2007b).

NCT is generally a cheaper production technology than ACT as the process does not require specialised equipments for aeration and can run without energy/electricity input, and is also considered to be more effective in controlling disease in most studies than the ACT (Welke, 2005; Scheuerell and Mahaffee, 2006; Scheuerell and Mahaffee, 2002; Koné *et al.*, 2010; Brinton *et al.*, 2004; Brinton and Droffner, 1995). Therefore, the cost/benefit of using NCT is more attractive and therefore NCT is of high relevance in low input agricultural systems, which include small to urban horticultural settings, if its suppressive potential is further evaluated and/or improved. Therefore, it is very important to evaluate the NCTs as a disease suppressant through varying production conditions and testing its efficacy in the pathosystem in question to generate adoptable technology with lesser cost and an easier fermentation process.

## 2.4. Factors affecting the quality and efficacy of NCTs

A number of factors involved in the production of NCT have a direct influence on both their microbiological and physico-chemical properties and hence on their efficacy as crop protectants. The type of compost feedstock is the most important factor. Variations in compost source have been shown to influence the level of disease suppression in many findings reported previously. This variation primarily stems from the nature of composition

of composting substrates. Some composting substrates are animal derived wastes whereas in some other feedstocks are plant-derived substrates as a source of compost tea making. Respective NCT sources have shown variable level of plant disease suppression in different studies reported in literature. For example, Dionne *et al.* (2012) reported that necrosis of tomato seedlings due to inoculation by *Pythium ultimum* was reduced by 100 % when applying NCT from bovine manure compost source whereas shrimp compost based NCT resulted in reduction by 58 %. Other NCT sources: chicken, sheep, and cow manure compost resulted in variable values ranging between 58-100 % control efficacies. Similarly, sheep manure, bovine, and shrimp based NCT by Koné *et al.* (2010) provided the highest inhibition of mycelial growth of *A. solani*, *B. cinerea*, and *P. infestans* of tomato in *in vitro* trials compared to two other NCT sources: chicken manure and seaweed compost .

Similarly, sorted solid municipal wastes have been studied in the past as a source of compost teas for use as bio- fertilizer. NCT made from composted household solid waste was evaluated as a foliar spray to study the mineral element uptake and fruit quality of raspberries by Hargreaves *et al.* (2008). NCT treatment increased leaf Na content compared to compost amendments itself, which suggest that foliar spray of NCTs are suitable as bio-fertilizer to channel plant nutrients.

In another study by Marin *et al.* (2013), NCT from spent mushroom compost and grape marc compost significantly reduced the severity of gummy stem blight (*Didymella bryoniae*) disease, whereas NCTs from greenhouse horticultural crop residues compost, vermicompost, and ACTs from the same compost sources did not exhibit effective control. The authors found that the plant waste based compost sources had greater bacterial than fungal populations and concluded that there existed a relationship between the level of pathogen



inhibition and the presence of culturable bacteria or fungi. It is however, arguable that culturable bacterial and fungal populations have solely contributed to the disease suppression given the existence of highly diverse unculturable microbes in compost tea.

These studies indicated that variability in compost source had a significant effect on disease suppression and such variation results in a difference of intrinsic characteristics of compost samples mainly physico-chemical and microbiological features that have direct influence on compositions of NCTs. Therefore, source compost type in terms of composition is always an important factor of NCT effectiveness improvement research.

Different parameters are stated in literatures that indicate other aspects of compost source and NCTs qualities. Some parameters influence the composting process whereas others have direct influence on the properties of NCTs during fermentation and therefore are interrelated. Temperature is one of physico-chemical parameters that influences the biological and chemical composition of compost and eventually the final product; both the solid particulate compost and the NCTs. For example, in any method of composting, the following three phases occur; (1) the mesophilic phase, up to a temperature of 40°C (2) the thermophilic phase, compost temperature >40°C, and (3) the cooling and maturation phase, a decrease in compost temperature by value of  $\pm 3^\circ\text{C}$  of ambient temperature (St. Martin and Brathwaite, 2012). These different stages are characterised by distinct compositions of microorganisms and concentration of chemical nutrients.

Similarly, for the NCTs, pH, electric conductivity (EC), and concentration of extractable cations and anions have been the major factors associated with compost and NCT qualities from a disease protection perspective. These have been considered to develop guidelines to produce suppressive NCTs. For example, fermentation temperature in the range 18–21°C

(Scheuerell and Mahaffee, 2002) is optimum for the growth of tea microbes during fermentation, while neutral pH and EC of less than 5 dS m<sup>-1</sup> are needed for microbial survival (Adegunloye *et al.*, 2007; Urban and Tränkner, 1993; Shrestha *et al.*, 2011b). In rare cases, attempts to vary these parameters have resulted in variable level of disease suppression (Yohalem *et al.*, 1996).

The age of compost and the process of composting have also been shown to result in NCTs with variable quality as a disease suppressant or as bio-fertilizer. In addition to compost to water ratio during the NCT making process, have been some other factors that are known to influence the quality of NCTs and hence its disease suppressive efficacy (Shrestha *et al.*, 2011b; Scheuerell and Mahaffee, 2002; 2006).

In addition to conventional composting, where temperature plays a major role for successive colonization of decomposing matter (Ryckeboer *et al.*, 2003a), and hence influencing the compositions and quality of resultant compost teas (Scheuerell and Mahaffee, 2002; St. Martin and Brathwaite, 2012), vermicomposting, which involves the bio-oxidation and stabilization of organic material by the joint action of earthworms and microorganisms, has also been shown in some studies as a source of good quality compost tea (Pant *et al.*, 2012). Microorganisms degrade the organic matter biochemically, whereas the earthworms are the crucial drivers of the process, as they aerate, condition and fragment the substrates, thereby drastically altering the microbial activity (Lazcano *et al.*, 2008). There is evidence that application of vermicompost improves the physical, chemical and biological properties of the soil and thus promotes and increases germination, growth and the yields of horticultural crops such as tomatoes, peppers, strawberries, foliage crops (Litterick *et al.*, 2004; Zaller, 2006). Compared to conventional thermophilic based NCTs,

little is known about the effect of vermicompost based NCTs as disease suppressants. Zaller (2006) found that only half as many plants sprayed with vermicompost were infected with *P. infestans* as water sprayed tomatoes. The author hypothesised that vermicompost tea spraying had altered the concentration of tissue N concentration to the point that leaves were resistant to the pathogen. However, susceptibility of leaves of plants due to the changes in concentration of nitrogen, as shown by Hoffland *et al.* (2000), is a condition of leaves that depends on the specific pathogen and therefore might not fully indicate the mechanism of suppression for the vermicompost tea used in the study. From the NCTs stand point, the assumption is that there is similarity in the types of factors, such as physico-chemical and microbial components, that influence disease suppressant efficacy of vermicompost based NCTs with that of conventional compost sources as indicated by Edwards *et al.* (2004).

## 2.5. Mechanisms of disease suppression by NCT

The exact mechanism of disease suppression by NCTs is not clearly understood. Multiple mechanisms have been proposed to explain the outcomes of the disease suppressive abilities of compost teas in trials conducted in the past. Both biotic and abiotic components are generally considered important (Naidu *et al.*, 2012; Mahaffee *et al.*, 2006; Hoitink and Changa, 2002) and therefore, disease suppression is a result both components of NCTs. The biotic components, mainly bacteria and fungi, may act as antagonists to the pathogen (Segarra *et al.*, 2009), compete for nutrients/space (microbiostasis) and reduce the growth of the pathogen (Hoitink and Changa, 2002) or suppress plant disease through inducing primed systemic resistance (Sang and Kim, 2011). Numerous reports have concluded that biotic components of compost tea play the main role and that disease suppression is a

microbially mediated phenomenon (El-Masry *et al.*, 2002; Koné *et al.*, 2010; On *et al.*, 2015b; Scheuerell, 2004; Siddiqui *et al.*, 2009; Haggag and Saber, 2007). For example, non-sterilized, filter-sterilized and heat-sterilized compost teas were tested on the growth of *C. cucurbitarum*, by Siddiqui *et al.* (2009) and found that the inhibitory efficacy was reduced significantly when the teas were subjected to millipore membrane filters or heat-sterilization. Application of both NCTs and ACTs effectively suppressed incidence of early and purple blight in tomato and onion, respectively as reported by Haggag and Timmusk (2008). Disease suppressive effect of crude NCTs was higher than that of the ACTs of the same compost sources. In addition, the application of filter-sterilised NCTs did not result in disease reduction suggesting that microbial cells were important for the suppressive effect. The authors quantified the microbial populations in terms of culturable microbes and the suppressive NCTs contained high numbers of actinomycetes, bacteria, filamentous fungi and yeasts. Applications of suppressive NCTs stimulated the induction of systemic resistance in hosts with an increase in chitinase,  $\beta$ -1, 3-glucanase and peroxidase activities. Therefore, they concluded that live microorganisms in the NCTs have played suppressive role by enhancing the disease resistance.

In an effort to understand the mechanisms of microbially mediated disease suppression, studies have been conducted on some aspects of culturable microorganisms. For example, the following authors found that the size of the populations of culturable microorganisms did not necessarily relate to the degree of disease suppression (Pane *et al.*, 2012; Scheuerell and Mahaffee, 2006; Palmer *et al.*, 2010). Scheuerell (2004), hypothesized that  $10^6$  c.f.u. ml<sup>-1</sup> of culturable bacteria represents transition between non-suppressive and suppressive compost teas. However, this hypothesis was contradicted by Pane *et al.* (2012), who found

that compost tea with  $10^3$  c.f.u. ml<sup>-1</sup> culturable bacteria did significantly inhibit disease caused by *Botrytis cinerea*, *Alternaria alternata* and *Pyrenochaeta lycopersici* on tomato. Similarly, in an experiment conducted by Scheuerell and Mahaffee (2006), increasing the population of culturable bacteria beyond this threshold limit by the addition of nutrients during fermentation did not result in a reduction of gray mold under the experimental conditions tested. Therefore, apart from the role of specific populations of culturable microbes, multiple mechanisms including unculturable microorganisms or diverse communities should be involved in disease suppression. Understanding the ecological structure of communities, in terms of microbial dominance, richness and composition, of compost tea may partly answer the questions of mechanism of suppression.

Numerous studies have also highlighted the potential role of abiotic factors in the suppressive abilities of compost teas. Microbial antibiotics and metabolites (Al-Dahmani *et al.*, 2003; Cronin *et al.*, 1996; Koné *et al.*, 2010) and nutrients that are released during biodegradation of the parent compost (Tang *et al.*, 2003), have been reported as factors that affect the suppressive efficacy of compost teas. For example, Cronin *et al.* (1996), elucidated that antibiosis was the mechanism leading to inhibition of *in vitro* conidial germination of *Venturia inaequalis* by spent mushroom NCT. The NCT produced in this study had suppressive activity after filtration and autoclaving due to the production of heat stable, non-protein metabolites of microorganisms during NCT fermentation. Chemical nutrients that are extracted into compost tea from compost mineralization have also been shown to influence the disease suppressive properties. The levels of nutrients such as NO<sub>3</sub>-N, NH<sub>4</sub>-N, and the C: N ratio were shown to indicate the stability, age, and biological properties of the parent compost materials (Danon *et al.*, 2007). In an experiment conducted to decouple the

role of abiotic components, Zmora-Nahum *et al.* (2008) reported that non-cured composts were found to completely inhibit sclerotia germination whereas extracts of cured compost allowed germination. They concluded that the concentration of nitrate is an important abiotic component to inhibit pathogen growth. However, it appeared that the condition was specific to *Sclerotia*, as other opportunistic microbes such as *Penicillium* and *Petriella* colonized the extracts despite the threshold concentration of the NO<sub>3</sub>.

## 2.6. The role of adjuvant addition to NCT

In microorganism mediated plant disease control, colonization and persistence of the microbes on the plant surfaces is an important process. Introduced biocontrols or a community of beneficial microbes are expected to fully colonise leaves to competitively antagonise the growth of or deter the initial landing of the pathogen. In most cases, however, colonization and relocation on the surface is challenging because of the hydrophobic nature of waxy leaves and sometime bacteria are unable to firmly attach and are washed off in severe weather (Crane and Bergstrom, 2014). To improve their ability to colonize and adhere to plant surfaces and thereby increase the efficacy of microbial antagonists, certain techniques have been developed. The addition of natural plant compounds in the form of nutrients or additives such as methyl jasmonate (Yao and Tian, 2005), essential oils (Arrebola *et al.*, 2010), tea tree saponin (Hao *et al.*, 2011), salicylic acid, gibberellic acid or beeswax (Sharma *et al.*, 2009), to microbial formulations can enhance the efficacy of biocontrols, partly by stimulation of the antagonistic population (Hao *et al.*, 2011) and partly by assisting the formation of microbial biofilm (Bais *et al.*, 2004) to aid effective colonization of leaf surface. It is recognized that the extent of adherence of biological control agents to the leaf surface could partly be due to biofilm formation. Biofilms are

multicellular communities in which cells are held together by an extracellular matrix that is composed mainly of exopolysaccharides, proteins and nucleic acids and other non-cellular materials (Zerrouh *et al.*, 2014; Danhorn and Fuqua, 2007). Emerging knowledge of microbe based plant disease control shows that biofilm formation helps enhance colonization and distribution of beneficial microbes over the plant surface (Bais *et al.*, 2004; Bogino *et al.*, 2013; Collins *et al.*, 2003).

Compost teas can be considered as a way of introducing a complex mixture of microorganisms to a plant surface (St Martin, 2015), and in previous studies variable degrees of colonisation of the plant surfaces by compost tea microorganisms relative to the pathogens have been indicated (Scheuerell and Mahaffee, 2006; Sturz *et al.*, 2006) . A few studies have been conducted to determine how to enhance the efficiency of leaf colonization by the compost tea microbes to enhance their efficacy as biocontrols. For example, the addition of natural products such as casein, pine oil (Ketterer *et al.*, 1992), and a polysaccharide gum karaya (Scheuerell and Mahaffee, 2006) to NCTs as spray adjuvants were shown to enhance colonization of microbes on the plant surface thereby resulting in better disease suppression.

Biopolymers have been reported to suppress plant disease either applied alone (Luiz *et al.*, 2012) or in combination with biocontrol agents to improve the degree of disease suppression (Lima *et al.*, 2005). Of the roles of biopolymers in biological-based plant disease management, their use as carriers in formulations (Chen *et al.*, 2013a) and as adjuvants whereby they slow the rate of microbial desiccation and stabilize the activity of microorganisms (Hynes and Boyetchko, 2006; Lima *et al.*, 2005), has been a growing area of research recently. However, there is a knowledge gap about role of additives and their

effect, particularly when an array of microorganisms, as is present in compost tea, are involved in disease suppression.

## 2.7. Summary

Research on utilization of compost tea as a tool for crop disease management has been a central area of investigation in organic and low-input agriculture. There are two methods of producing compost tea production: aerated (ACT) and non-aerated compost tea (NCT) based on the presence or absence of oxygen during the extraction process. The NCTs are regarded as lower in production cost, using simplistic methodology, while additionally being more effective in the wide range of pathosystems tested. This literature review has highlighted the magnitude of disease suppression of NCTs, which is influenced by many factors. Apart from the nature of the pathosystem in question, the inherent physico-chemical and microbiological characteristics of the parent compost, has been stated as a factor that dictates the suppressive abilities of NCTs. Variation in compost age, the compositions of compost substrates, methods of composting such as conventional and vermicomposting have all influenced the effectiveness of NCTs. Finally, the use of adjuvants seems a promising approach that should be explored further to determine its role in enhancing the efficacy of NCTs.



# Chapter 3

## FACTORS AFFECTING EFFICACY OF COMPOST TEA AGAINST SELECTED FUNGAL PATHOGENS OF POTATO

### 3.1. Abstract

The aim of this study was to investigate key factors (including compost type and concentration) of two non-aerated compost teas and mechanisms that influence the restriction of selected fungal potato pathogens (two *Alternaria* spp. and *Rhizoctonia solani* isolates). Two non-aerated compost teas (NCTs, from commercial compost, CCT and vineyard compost, VCT) were tested for their ability to restrict mycelial growth of potato pathogens using *in vitro* assays and CCT was tested using detached leaf assays. The living microbial component of the tea was essential for suppression of potato pathogens *Alternaria solani* and *Rhizoctonia solani* in *in vitro* studies. In these studies, the VCT was generally more suppressive than CCT, with highest inhibition of *A. solani* mycelial growth (90% cf. 74% for VCT cf. CCT) and *R. solani* 299 (60% cf. 45% for VCT cf. CCT). However, CCT led to greater inhibition of *R. solani* 422 (84% cf. 82% for CCT cf. VCT). The application concentration altered efficacy in a different manner for each type of NCT. Microbial diversity (based on 16S rRNA and ITS gene sequences) revealed that the CCT had higher fungal and bacterial diversity and richness than the VCT. Use of CCT significantly reduced lesion area of *Alternaria alternata* on detached leaves, however plant derived gum as an

adjuvant to CCT did not lead to significantly greater control. Scanning electron microscopy showed that the spatial distribution of microbes from the CCT was altered with gum addition, to resemble what may have been a microbial biofilm. We confirmed that suppression of fungal pathogen growth *in vitro* by NCTs depends on the live microbial component. Each NCT could suppress the mycelial growth of selected potato pathogens in culture and CCT reduced *A. alternata* lesions on detached potato leaves. Factors involved in efficacy (concentration, microbial communities, physico-chemical properties) could not be consistently linked to NCT efficacy. This study shows the potential for NCT's as a crop protection tool to reduce growth of selected potato pathogens, with up to 90% fungal growth inhibition *in vitro* and reduction of lesions by up to approximately half in a detached leaf assay. It documents the relative abundance of specific microbial taxa associated with pathogen and disease-suppressive NCTs, both at phylum and genus level, and provides images of the spatial distribution of NCT microbes on plant surfaces relative to the colonising pathogen.

### 3.2. Introduction

Compost tea represents an alternative biocontrol approach to manage plant diseases, where multiple microorganisms are simultaneously applied to control one or more pathogens rather than using a single biocontrol species (St Martin, 2015; Evans and Percy, 2014). The mechanism of disease suppression by compost teas is still not clearly understood. To explain how the application of compost tea leads to significant suppression of plant disease, multiple mechanisms have been proposed, including the presence of bacteria and fungi in the compost tea, which may act as antagonists to the pathogen (Segarra *et al.*,

2009), compete for nutrients/space (microbiostasis) (Hoitink and Changa, 2002) or results in treatment responses that are consistent with primed systemic resistance (Sang and Kim, 2011; Evans *et al.*, 2013).

While some reports have highlighted the potential role of abiotic factors, including nutrients in the compost tea (Tang *et al.*, 2003) or microbial antibiotics (Mahaffee *et al.*, 2006; Koné *et al.*, 2010; Al-Dahmani *et al.*, 2003), biotic components have generally been regarded as critical in mediating the mode of action. For example, compost tea efficacy was significantly reduced (Siddiqui *et al.*, 2009) or lost (Gea *et al.*, 2009) after autoclaving or filtration, suggesting that live microorganisms in compost teas have a direct effect.

Therefore, central to understanding disease suppression is a need to understand the compost tea microorganisms, including their diversity and distribution on plant surfaces post-application and their interaction with the pathogen.

In conventional biological control, the goal is to select microbial antagonists that are better adapted to environmental and nutritional conditions than the pathogen to ensure they colonise the plant more rapidly than the pathogen (El Ghaouth *et al.*, 2004). However, their efficacies are mostly inconsistent due to complex interactions among host, pathogen, antagonist, and the range of environmental conditions encountered (Weller, 1988; Berg *et al.*, 2006; Berg, 2009). Over the years, research has been conducted to improve the efficacy of antagonists and to enhance their ability to colonize and adhere to plant surfaces. Likewise, addition of natural plant compounds in the form of nutrients or additives including methyl jasmonate (Yao and Tian, 2005), essential oils (Arrebola *et al.*, 2010), tea tree saponin (Hao *et al.*, 2011), salicylic acid, gibberellic acid or beeswax (Sharma *et al.*, 2009), to microbial formulations can enhance the efficacy of biocontrols, partly by stimulation of the

antagonistic population (Hao *et al.*, 2011) and partly by the formation of biofilms which may aid microbial colonization of the plant surface (Bais *et al.*, 2004; Beauregard *et al.*, 2013; Chen *et al.*, 2013b). Biofilms are assemblages of microorganisms adherent to each other and to a surface and are embedded in a matrix of extracellular polymeric substances that is composed of exopolysaccharides, proteins and nucleic acids and other non-cellular materials (Zerrouh *et al.*, 2014; Danhorn and Fuqua, 2007; Morris *et al.*, 1997).

Previous studies indicated that the culturable microbes from compost tea exhibited different degrees of colonization of plant surfaces compared to the disease causing pathogen (Andrews and Harris, 1992; Sackenheim *et al.*, 1994). In field experimentation to suppress powdery mildew on leaves of Chardonnay grapevines, for example, Evans *et al.* (2013) reported that aerated compost teas (ACTs) were associated with an increase in culturable microbes for the first three weeks after application, but after that the number of microbes started to decline. Similarly, following application of compost teas on potato leaves by Sturz *et al.* (2006), microbial communities were poorly established on the phylloplane, either due to a lack in competence or to being washed off during spraying of compost tea, and thus had less antibiosis activity against *Phytophthora infestans* as compared to the *in vitro* efficacy of the culturable fraction in the tank mixtures prior to application. Therefore, the extent to which compost tea microbes establish on leaf surfaces could partly influence the effectiveness to suppress the growth of the pathogen in question or deter its establishment.

Competitive colonization of leaf surfaces for microbe-mediated disease control could be enhanced if modification of the leaf environment is attained. Scheuerell and Mahaffee (2006) indicated that modifying the phylloplane environment by adding an adjuvant such as

plant derived gum or other polysaccharides could enhance the adherence of compost tea droplets and thus colonization of microbes on the leaf surface. Several studies have been conducted to investigate adjuvants for enhancing the biocontrol efficacy of compost teas. Addition of natural products such as casein, pine oil (Ketterer *et al.*, 1992), and gum karaya and other polysaccharides (Scheuerell and Mahaffee, 2006) to NCTs as spray adjuvants were shown to enhance colonisation of the plant surface by the compost tea microbes, thereby resulting in greater disease suppression. There have not been, however, reports that explain the possible role of such additives and their effect on the interaction of microbial communities with the pathogen and/or overall role in disease control efficacy.

Both the soil borne potato disease black scurf/Rhizoctonia canker caused by *Rhizoctonia solani* and foliar early blight disease caused by *Alternaria solani* are economically important problems for the potato industry with significant yield and quality losses reported across Australia (Sparrow and Wilson, 2012). Brown leaf spot disease caused by *A. alternata* is a destructive and common disease of the cultivated potato and infection is facilitated by wounding. This disease progressively weakens the plant and increases its susceptibility to infection by reducing the photosynthetic leaf area and increases the imbalance between nutrient demand of leaves (Soleimani and Kirk, 2012). In addition to cultural practices, a wide range of fungicides are available to aid control of these fungal diseases in conventional programs (Horsfield *et al.*, 2010; Wicks *et al.*, 1996). Organic growers, however, do not have enough available options for control of fungal diseases of horticultural crops in general (Van Zwieten *et al.*, 2007a) and low-cost biological options such as compost tea could provide an alternative if sufficiently effective.

To support development of such alternatives for disease control in potato, we explored key questions related to the efficacy and mode of action of NCTs, using a soil borne root and tuber pathogen (*R. solani*) and two foliar pathogens (*A. solani* and *A. alternata*) and two compost sources. The first objective of this study was to evaluate the importance of the biotic component (i.e. microorganisms) of NCTs, at a range of concentrations, using an *in vitro* bioassay. We hypothesized that the ability of the NCT to restrict growth of the fungal pathogens tested would be greater in the presence of the biotic component of each NCT assayed. The second objective was to investigate the role of additives on the efficacy of NCTs and their effect on the spatial arrangement of microorganisms on the surface of potato foliage assessed using scanning electron microscopy (SEM) and environmental SEM (ESEM) of detached leaves. We hypothesised that the additives would enhance efficacy of the NCTs to control disease and that spatial arrangement of microorganisms would be altered by their addition to the NCTs.

### 3.3 Materials and Methods

#### *Compost sources and non-aerated compost tea*

Mature composts were obtained from southern Tasmania. A vineyard derived compost (VC) and commercially produced organic compost (CC) were used. The compost source material and composting methods are shown in Table 1. The non-aerated compost teas (NCTs) were extracted according to the procedure outlined by Koné *et al.* (2010). Each batch of compost tea was prepared in a clean plastic bucket (20 L capacity) in a 1:5 ratio (w/v) of air-dried compost (dry weight) basis to dechlorinated water. The mixture was then allowed to ferment for a period of 14 days at 18 °C in the dark. A layered muslin cloth was used to filter the bulk compost material of the respective compost teas. Single batches of NCTs were used

in subsequent experiments within a period of 2-3 weeks. During this period, the NCTs were stored at 4 °C in the dark.

**Table 1** Compost types used in this study; starting materials and composting method.

Compost	Starting materials	Composting method	Age
<b>Vineyard (VC)</b>	Winery waste, apple waste, blackcurrant and raspberry waste, wood chips, quarry fines, poultry manure and garlic waste	Open windrow, max temp 65 °C, turned if CO <sub>2</sub> above 14 ppm.	Mature
<b>Commercial (CC)</b>	Fish waste, chicken litter, food scraps and organic sludge	Open windrow composting CO <sub>2</sub> testing every 12 weeks	Mature

Physico-chemical characters of the parent compost and the compost teas were measured during the start of the experiment. For the NCTs, electric conductivity (EC), pH, and range of extractable cations and anions were measured. C: N ratio and measures of extractable cations and anions concentrations were determined for the parent compost sources, as shown in Table 2.

**Table 2** Physico-chemical measurements of parent compost and compost teas used in the bioassays.

Parameters	Units	Compost sources*	
		VC	CC
C:N		12.8	13.2
Total Nitrogen	(%)	2.32	0.98
Carbon	(%)	29.8	12.9
Mg	(%)	0.38	0.33
K	(%)	1.75	0.69
Na	(%)	0.11	0.19
Ca	(%)	1.38	3.02
NO <sub>3</sub>	(mg/Kg)	116.3	734.08
Fe	(mg/Kg)	7090	18290
Mn	(mg/Kg)	295.2	954.2
Zn	(mg/Kg)	141.8	156.1
		Compost teas*	
		VCT	CCT
pH		7.6	7.5
EC	(dS/m)	2.26	2.69
Mg	(mg/L)	17.93	27.81
K	(mg/L)	783.7	491.6
Na	(mg/L)	45.76	150.7
Ca	(mg/L)	34.95	46.93
NO <sub>3</sub>	(mg/L)	1.02	102.28
NH <sub>4</sub> N	(mg/L)	20.97	1.46
Fe	(mg/L)	5.82	1.09
S	(mg/L)	20.83	46.07
B	(mg/L)	0.91	0.32

\*Data was obtained from a single sample of compost and single batch of each tea.

### *Microbial diversity of the NCTs*

Microbial communities of both NCT types were examined using a batch of each NCT made after the *in vitro* and detached leaf assay trials, but with identical preparation method.

Metagenomic DNA was extracted in triplicate using the Power Soil DNA kit (MO BIO

Laboratories, Inc., USA) according to the manufacturer's instructions. A volume of 30 ml of compost tea was centrifuged (2900 x g for 30 min) and up to 0.3 g of the pellets were used

for extraction of DNA. Purity was measured using the spectrophotometer at wavelengths of 260/280 nm (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington,



DE, U.S.A.). The DNA concentration in the extracts ranged from 5 – 15 ng/μL and the A260:280 ratio ranged from 1.6 – 2.0. High throughput amplicon sequencing was conducted according to the methods described by Caporaso *et al.* (2011). Sequencing was performed at MR DNA (Shallowater, TX, USA) on the Illumina MiSeq platform following the manufacturer's guidelines. The V4 variable region of the 16S rRNA gene (for bacteria and archaea) was amplified with the PCR primers 515F/806R and the internal transcribed spacer region (ITS1) of the nuclear ribosomal RNA gene (for fungi) was amplified with primers ITS1-F/ITS2. Amplifications were performed in a 30 cycle PCR using the Hot Star Taq Plus Master Mix Kit (Qiagen, USA). The PCR conditions were: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. The products of multiple PCRs were pooled together in equal proportions based on their molecular weight and DNA concentrations and purified using calibrated Ampure XP beads. These PCR products were used to prepare a DNA library following the Illumina TruSeq DNA library preparation protocol. Sequence data were processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, paired sequences were joined, barcodes removed, and sequences containing ambiguous base calls, and with a length < 150 bp or suspected chimeras were removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes (DeSantis *et al.*, 2006), RDP11 and NCBI ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov), <http://rdp.cme.msu.edu>).

Two strains of *Rhizoctonia solani* AG3-PT (R299 and R422) and a strain of *Alternaria solani*, isolated originally from potato (*Solanum tuberosum* L.), were obtained from a collection of fungal isolates at New Town Research Laboratories, Hobart. A pathogenic strain of *Alternaria alternata* was isolated and identified from foliage of a commercial potato crop of the Russet Burbank cultivar showing brown spot symptoms, growing in Sassafras, North West (NW) coast area of Tasmania. The leaf samples were surface sterilized with 1% sodium hypochlorite for 1 min, cut into pieces and separately transferred to potato dextrose agar (PDA) and incubated at 25°C for about 14 days. Microscopic examination, sporulation abilities and the morphological features of isolates were examined. DNA extraction and sequencing of six genes were conducted to confirm the identity of the fungal pathogen. All the fungal isolates were maintained on potato dextrose agar media (PDA) at 4 °C and were occasionally subcultured to maintain active culture growth.

#### *In vitro* mycelial growth inhibition

The efficacy of filtered or non-filtered NCT in suppressing the mycelial growth of the pathogens *R. solani* AG3-PT (R299 and R422) and *Alternaria solani* was tested according to the procedure outlined by Diáñez *et al.* (2006). The two types of NCTs (VCT batch 1 and CCT batch 1) from the two compost sources (VC and CC) were used either as crude NCT or after sterilization by filtration using an 0.2 µm filter, and were then incorporated at four concentrations: 5%, 10%, 15%, and 20% (v/v) in the potato dextrose agar (PDA) media to be used subsequently to assess pathogen growth. Each NCT (filtered or not) was mixed with autoclaved PDA once cooled to 45 °C. Circular agar plugs (5 mm in diameter) covered with actively growing, 14-day old mycelia of the respective fungal pathogens were prepared with a sterile cork borer and placed in the centre of 9 cm-diameter Petri plates containing PDA-

NCT medium and incubated for 6–7 days in the dark at 25 °C. PDA plates with no NCT but containing the plugs of the respective pathogens were used as controls. High definition images of the reverse side of the agar plates were obtained with an automated imaging instrument (ProtoCOL 3, Synbiosis, UK). This instrument housed an internal high resolution CCD camera, along with a LED lighting configuration system. Radial mycelial growth of each fungal culture was measured perpendicularly by Image J software (Schneider *et al.*, 2012) and the mean of two measures was determined. The experiment was conducted according to a completely randomized design with four replicates and repeated once.

#### *Detached leaf assays – experimental design and visual assessment*

Two detached leaf bioassay trials were conducted with *A. alternata*. Leaf material was sourced from 2.5 month-old potato plants (Russet Burbank) grown in a glass house.

*Alternaria solani* was observed to be poorly pathogenic in preliminary study and was not considered further in detached leaf assay. In both trials, the CCT and an isolate of *A. alternata* were used. The first trial was conducted with CCT batch 2, which had been stored for 15 days after preparation. CCT was used rather than VCT as the compost is commercially produced from the same inputs and therefore likely to be more consistent in composition over time (Personal communication, Pure living Soils Compost, Hobart) while the vineyard compost is not made regularly. The aim of this trial was to determine if wounding was required to establish infection and whether wounding influenced the efficacy of NCT treatment. Detached leaves were rinsed with sterile water, dried in sterile conditions and then either wounded with sterile scalpel or not wounded prior to inoculation with 5 mm<sup>3</sup> of mycelial mass transferred to the abaxial surface of each leaf, according to the methods by Schuck *et al.* (2014), with modification. The mycelium was applied at two positions of each

leaf, on both sides of the mid-vein, and directly on the top of the wound (when wounded). After inoculation, 200 µl of either NCT or distilled water were added in drops on leaves. Each leaf was placed on water agar (15 g/l) in a Petri dish and incubated at 25 °C, approximately 60% RH and a 16 h photoperiod. The experiment was conducted in a 2 x 2 factorial design (Table 3) in which leaves were inoculated with or without wounding and with either application of CCT or sterile distilled water. Additionally one non-inoculated treatment in which leaves were treated with CCT but not wounded was included as a reference. Four replications (a total of 20 potato leaves) were arranged in a completely randomised design. Chlorotic and/or necrotic symptom development was examined after 7 days incubation.

A second trial examined the effect on *A. alternata* infection of detached leaves when CCT was applied alone or in combination with the spray adjuvant gum Arabic (G) (The Melbourne Food ingredient depot, East Brunswick, Vic), based on a concentration stated by Scheuerell and Mahaffee (2006) of 0.05 % w/v. This trial was conducted with CCT batch 2, which had been stored for 20 days after preparation. The gum was added in powder form several minutes before the CCT was applied, and was well mixed and dissolved. Potato leaves with no wounding were inoculated with a 5 mm disc of mycelium. Inoculation method, incubation conditions and volumes of CCT and sterile distilled water (control) were as in the first trial. The experiment was conducted with four replicates.

The same automated imaging instrument as used for the *in vitro* trial was used to obtain images of leaves from the detached assays. The background of each image (excluding the whole leaf area) was converted to blue to enhance further image analysis. The amount of leaf lesion as an indicator of blight severity of leaves were measured by image-based plant

disease quantification software ASSESS 2.0 (Lamari, 2002). Thresholds of leaf and lesions were set on the classic panel in the HSI (hue, saturation and intensity) colour space using saturation values of 31 to 131 that separate leaf from background and 31 to 108 to threshold the lesion so that both chlorotic and necrotic blight symptoms were included. Disease percentage was calculated as (lesion pixels / leaf pixels) \* 100. In cases where replicate measurements varied significantly using a classic threshold panel, a manual panel was used instead to take into account leaf and necrotic spot shape.

#### *Detached leaf assays - observation with ESEM and SEM*

After visual assessment of incubated leaves from the bioassay trials above, Environmental Scanning Electron Microscopy (ESEM) (FEI MLA650, Bruker Quantax) and Scanning Electron Microscopy (SEM) (High resolution Hitachi SU-70) were used to visualise the spatial arrangement of CCT microbes and the *A. alternata* pathogen on the leaf surfaces. The ESEM was operated in 'wet' mode with the large-field gaseous secondary electron detector. Selected specimens (lesion area of the leaf) were cut in small pieces, mounted on aluminium stubs using carbon-impregnated double-sided tape and immediately transferred to the microscope chamber.

For SEM assessment, leaves were first fixed using a glutaraldehyde solution and then dehydrated using procedures described by (Morris *et al.*, 1997), with some modification. Briefly, specimens were cut in to 1 mm<sup>3</sup> cubes and placed in fixative solutions containing 2.5% glutaraldehyde, plus 2% formaldehyde with 2 mM calcium chloride and 0.1 M sodium cacodylate buffer. Samples were then washed three times, postfixed for 1 h in 1% osmium tetroxide solution at room temperature, and then further stabilised for 30 min in a 1% aqueous solution of uranyl acetate at room temperature. Samples were then dehydrated

with an ethanol series through to 100% ethanol, after which the dehydrated leaf material was fixed on metal supports and sputter coated with platinum (10 nm) and observed by SEM.

#### *Data analysis*

For the *in vitro* study of mycelial growth, the percentage of inhibition was calculated relative to the radial fungal growth in the control plates. Analysis of variance (ANOVA) was carried out with the GLM procedure of SAS (SAS Institute, 2011) and, means were compared using Fisher's protected least significant difference (LSD) test when treatment effects were significant ( $P < 0.05$ ). For the detached leaf assays, the effect of treatments on lesion area as a percentage leaf area were analysed by ANOVA and means separated as described above.

### 3.4. Results

#### *Physico-chemical properties of the NCTs*

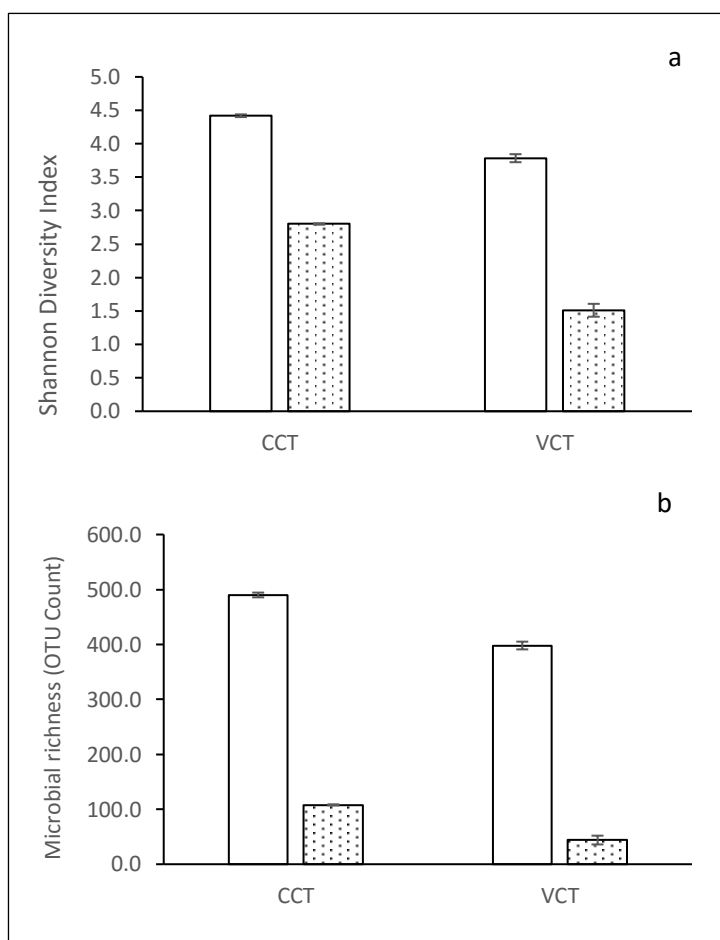
As only one sample of each NCT from a single batch was analysed, physico-chemical properties present trends only for reference. As a percentage of compost mass, the VC had more carbon and nitrogen, but the C: N ratio was similar (slightly higher for CC than the VC (Table 2). The form of nitrogen present in each compost contrasted greatly, that is, there was approx. 6-fold more nitrate ( $\text{NO}_3$ ) in the CC than the VC (Table 2). The CC also had over 2-fold more Fe and 3-fold more Mn and Ca (Table 2).

Both NCTs had similar neutral pH values whereas CCT had a higher EC (2.69 dS/m) than that of the VCT (2.26 dS/m) (Table 2). Except K and Fe, the concentration of all other cations and anions measured were markedly higher in the CCT than the VCT (Table 2). In a similar trend found for the composts, nitrate was much higher (100-fold) in the CCT compared to the VCT,

while the N in VCT was present in higher concentration of ammonium ( $\text{NH}_4\text{N}$ ) compared to CCT (Table 2).

#### *Microbial community structure and diversity*

16S rRNA and ITS gene sequence analysis revealed that the unfiltered compost teas harboured diverse bacterial and fungal populations (Fig. 1). The CCT had significantly higher values of the Shannon-Weaver diversity index (H) for both bacteria ( $p=0.0006$ ) and fungi ( $p=0.0002$ ) and richness (number of the OTUs) for both bacteria ( $p=0.0004$ ) and for fungi ( $p=0.0014$ ) than the VCT (Fig. 1). The numbers of bacterial OTUs and the Shannon diversity index in both NCTs were markedly higher than the corresponding values for fungi (Fig. 1).



**Figure 1** Diversity indices of NCT samples, CCT (Commercial compost tea) and VCT (Vineyard compost tea) based on the OTU sequences: a) bacterial and fungal Shannon diversity indices (H), b) bacterial and fungal richness in terms of number of OTUs. The error bars represent standard error (n=3). White bars are bacterial (16S) and stippled bars are fungal (ITS) data.

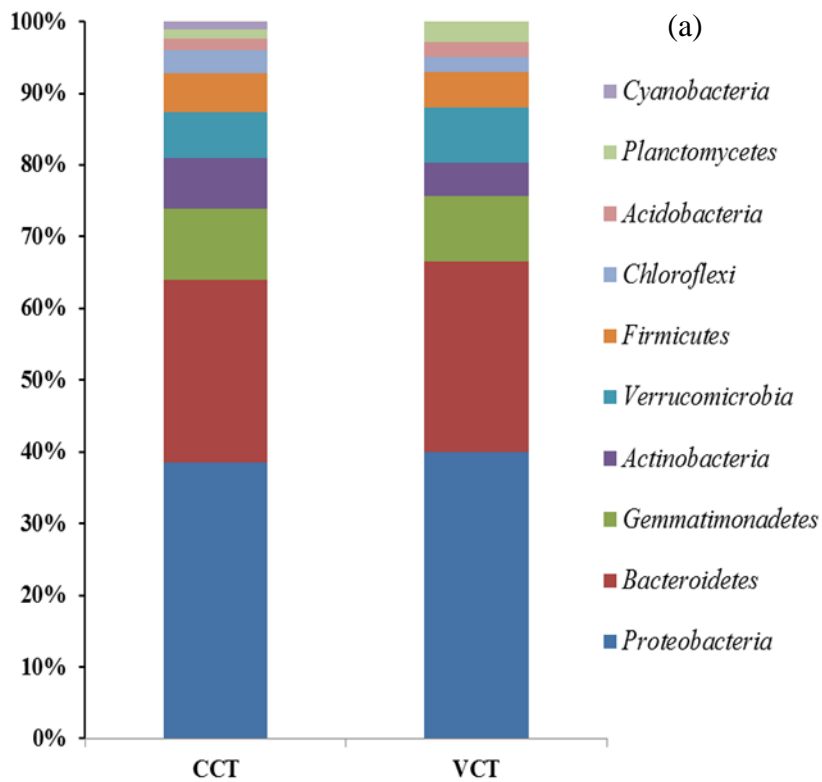
Both NCTs types had similar bacterial community compositions and had approximately the same proportions when sequence reads were classified at the phylum taxonomic level (Fig. 2). The main phyla present were *Proteobacteria* (39%), *Bacteroidetes* (25%), *Gemmatimonadetes* (10%), *Actinobacteria* (5%), *Verrucomicrobia* (7%) and *Firmicutes* (5%) (Fig. 2a). These phyla are commonly associated with environments such as soils, compost and water.

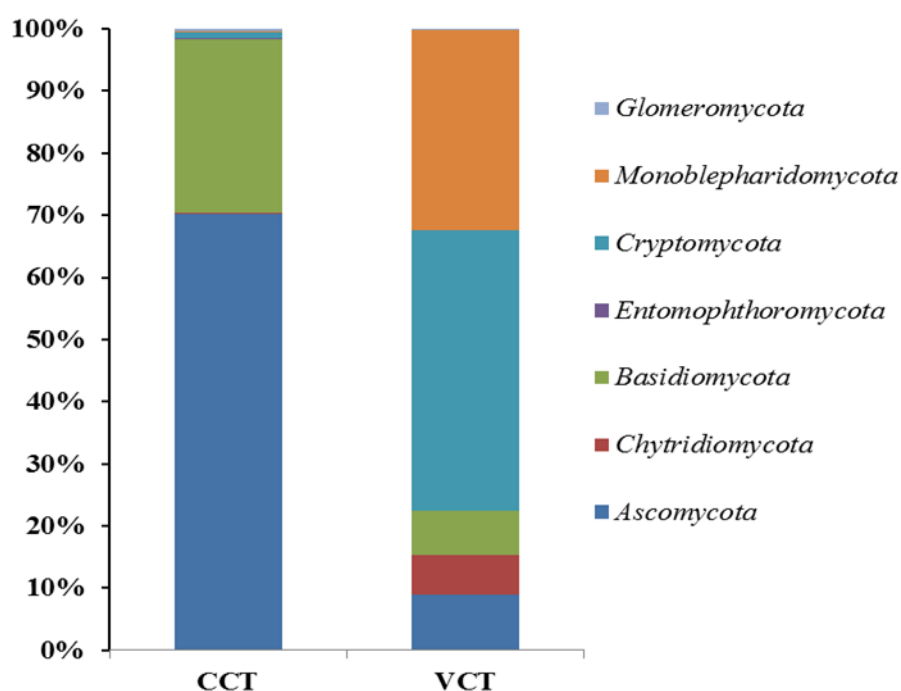
At the genus level classification, genera such as *Blastochloris*, *Cerasibacillus*, *Rhodocista*, *Paenibacillus*, *Hydrogenophaga*, and *Ilumatobacter* were present in both types of NCT although with different relative abundances (Figure S1). Genera known for their widespread presence in natural ecosystems, including *Nitrospira*, *Algisphaera*, *Caldalkalibacillus*, *Fodinicola*, *Runella*, *Larkinella*, and *Proteiniborus*, were only present in CCT (Figure S1).

The fungal community compositions in the two NCTs were markedly different both at the phylum (Fig. 2b) and genus level classification (Figure S2). The CCT consisted mainly of the phyla *Ascomycota* (over 70%) and *Basidiomycota* (28%) whereas the VCT contained *Cryptomycota* (45%), *Monoblepharidomycota* (32%), *Ascomycota* (9%) and *Basidiomycota* (7%) (Fig. 2b). At genus level, a wide range of genera were detected including *Myriococcum*, *Thermomyces*, *Chaetomium*, *Geomyces*, *Paramicrosporidium*, *Penicillium*, and *Trichoderma*, which were found in both NCTs in different relative abundances (Figure S2). Fungal genera in the CCT were generally diverse with no one genus dominating the community, whereas



VCT contained higher abundances of specific genera. For example, *Paramicrosporidium* and *Hyaloraphidium* combined constituted a relative abundance of more than 76% (Figure S2).

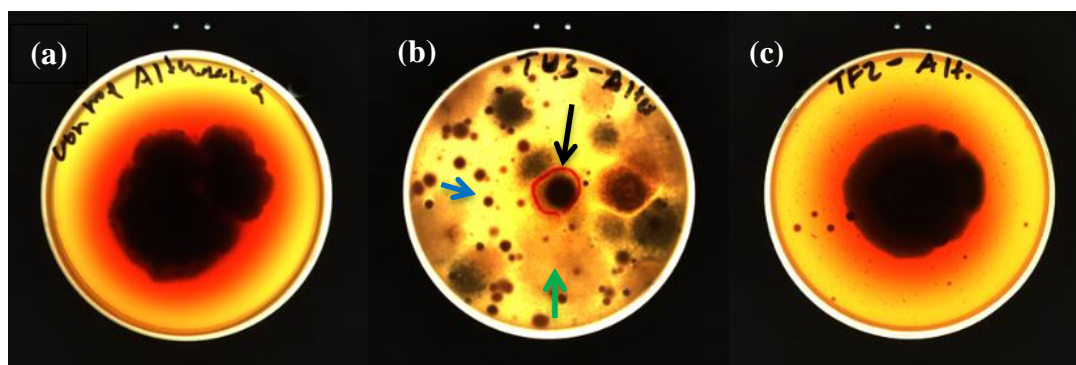




**Figure 2** Relative abundances expressed as percentage of the total number of sequences of the major bacterial (a) and fungal (b) phyla in both compost teas: CCT (commercial compost tea) & VCT (vineyard compost tea).

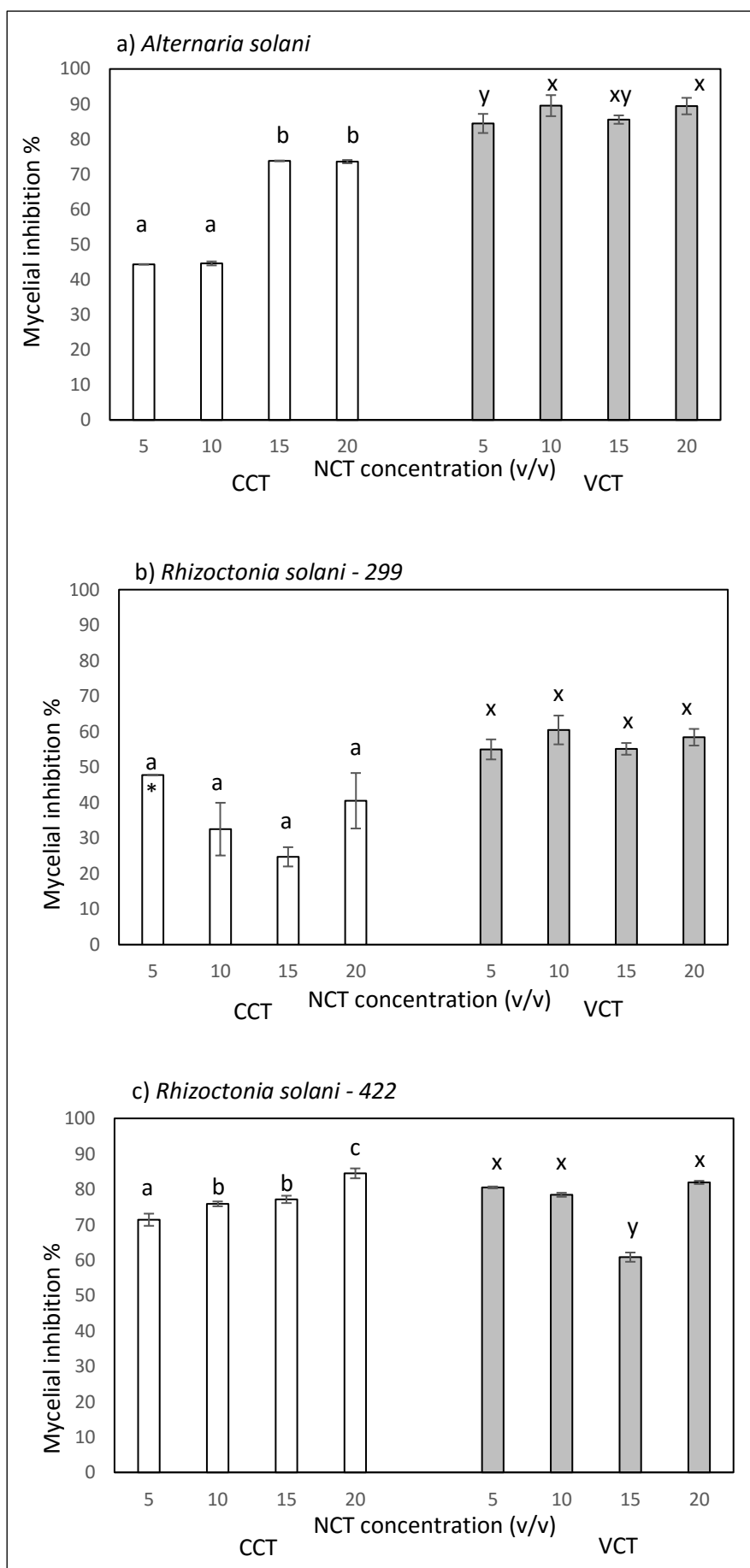
#### *Effect of NCT on mycelial growth*

Each experiment was conducted twice, however similar trends were observed in both experiments therefore the data for the first experiment only is presented. The *in vitro* assays revealed prominent differences in pathogen inhibition between unfiltered and filtered NCTs, as illustrated by example images of incubated plates (Fig. 3a-c). Filtration of either type of NCT resulted in no discernible inhibition of mycelial growth of any pathogen compared to the controls (data not presented, except Fig. 3c).



**Figure 3** The impact of compost tea microbes on radial growth of fungal mycelium. a) mycelium of *A. solani* on potato dextrose agar (PDA); b) reduced growth of mycelium of *A. solani* incubated with 15% of unfiltered compost tea (CCT) the black arrow indicates mycelium of *A. solani*, the blue arrow indicates bacterial colonies and the green arrow is fungal mycelia from the CCT); c) mycelium of *A. solani* incubated with 15% of filtered compost tea for 5 days.

Unfiltered NCT from both sources of compost and for all concentrations tested generally inhibited the radial growth of mycelia of all the three pathogens compared to control plates (Fig. 4a-c).



**Figure 4** The efficacy of two compost tea types, commercial compost tea (CCT) and vineyard compost tea (VCT), applied at different concentrations (5%, 10%, 15%, 20%) (v/v) on suppression of fungal mycelial growth. a) *Alternaria solani*, b) *Rhizoctonia solani* isolate 299-AG and c) *Rhizoctonia solani* isolate 422-AG. Bars represent the standard error (n= 5) except as shown by \* (n=1). Letters above each column which are different for either CCT (a-c) or VCT (x-y) indicate significant differences of mycelial inhibition due to NCT concentration.

For *A. solani*, there was a highly significant interaction ( $P<0.0001$ ) of the NCT type and concentration factors on the mycelial inhibition. For CCT, mycelial inhibition occurred in a concentration dependent manner (Fig. 4a). That is, there was a marked (but not linear) increase in efficacy as the CCT concentration increased, with inhibition increasing significantly (and almost two-fold) from the 5 and 10% concentrations, to the 15 or 20% concentrations (Fig. 4a). Application of VCT at all concentrations resulted in mycelial inhibition that ranged from 86% to 90%. For concentrations above 5%, the effect on pathogen inhibition percentage was similar in all the three remaining concentrations (10, 15, 20%).

In the case of *R. solani*-299, NCT type had a highly significant effect ( $P<0.0001$ ), but varying the concentrations of the compost teas had no effect on mycelial inhibition ( $P=0.47$ ), and nor did interaction between the two factors ( $P=0.42$ ). Regardless of application concentration, the VCT resulted in mean inhibition of 57% as compared to 36% for CCT (Fig. 4b). For the third test pathogen *R. solani*-422, there were no differences between the two types of NCT in the magnitude of inhibition ( $P>0.05$ ), but there was a highly significant interaction effect ( $P<0.0001$ ) between each NCT type and concentration on the growth of the pathogen.

Overall, inhibition of *R. solani*-422 mycelial growth ranged from 61% to 82% when VCT was used (Fig. 4c). The variation in concentration of VCT appeared to have no effect on the degree of growth inhibition of *R. solani*-422 mycelia except that VCT at 15% concentration resulted in significantly lower level of inhibition than the other concentrations. However, increasing the tea concentration level from 5% to 20% for the CCT enhanced the efficacy from 71% to 85%, a concentration dependent inhibition was observed, as inhibition in response to the 10 and 15% treatments was significantly higher than for the 5% and less than at 20% (Fig. 4c).

#### *Detached leaf assays – effect of NCT on infection by A. alternata*

In the first detached leaf assay trial, the results revealed no statistically significant interaction between wounding and CCT application on lesion area although the two factors were both significant at  $P < 0.05$  (Table 3). The largest percentage lesion area was measured for detached leaves which were inoculated and wounded but treated with water, while the same treatment without wounding resulted in significantly smaller lesion area (Table 3). Application of CCT reduced lesion size by approximately 2-fold for both wounded and non-wounded detached leaves (Table 3). As a reference, unwounded leaves were also subject to application with CCT but no inoculum was added; the mean lesion size was negligible at 0.8% measured as a percentage of leaf area, confirming that the lesions were due to the pathogen presence.

**Table 3** Mean fungal blight lesion area in response to commercial compost tea (CCT) application on detached potato leaves inoculated with mycelium\* of *A. alternata*, with and without wounding.

Treatments	Wounding	Application	% lesion <sup>†</sup>
1	Yes	Water	78.9 a
2	No	Water	58.4 b
3	Yes	CCT	47.9 bc
4	No	CCT	36.6 c
P value 0.0099			
P value (interaction of terms)			0.4995
P value (wounding)			0.0402
P value (application)			0.0037

\*Mycelial disc of 5 mm transferred on to the leaf abaxial surfaces from a 14-day-old culture of *A. alternata*;

<sup>†</sup>Lesion area as a percentage of leaf area was measured by ASSESS 2:0 software after incubation of leaves for 7 days at 25 °C and 60% relative humidity.

Within the column, values sharing the same letter are not significantly different.

In the second trial, the application of compost tea either alone or with spray adjuvant (gum arabic) on inoculated leaves resulted in restricted development of symptoms after 7 days of incubation compared to the controls (Table 4). Leaves that were not treated had significantly ( $P=0.009$ ) greater lesion area (approx. 40% greater) than those treated with CCT (Table 4). The addition of gum to the CCT did not lead to a significant difference in lesion area (Table 4).

**Table 4** Mean fungal blight lesion area in response to commercial compost tea (CCT) application, with and without gum, on detached, unwounded potato leaves inoculated with mycelium\* of *A. alternata*.

Treatments	Application <sup>‡</sup>	% lesions <sup>†</sup>
A	Water	58.2 a
B	None	56.7 a
C	CCT	38.8 b
D	CCT+Gum	42.3 b

\* Mycelial disc of 5 mm transferred on to the leaf abaxial surfaces from a 14-day-old culture of *A. alternata*;

<sup>†</sup>The lesion area as a percentage of leaf area was measured by ASSESS2:0 software after incubation of leaves for 7 days at 25 °C and 60% relative humidity.

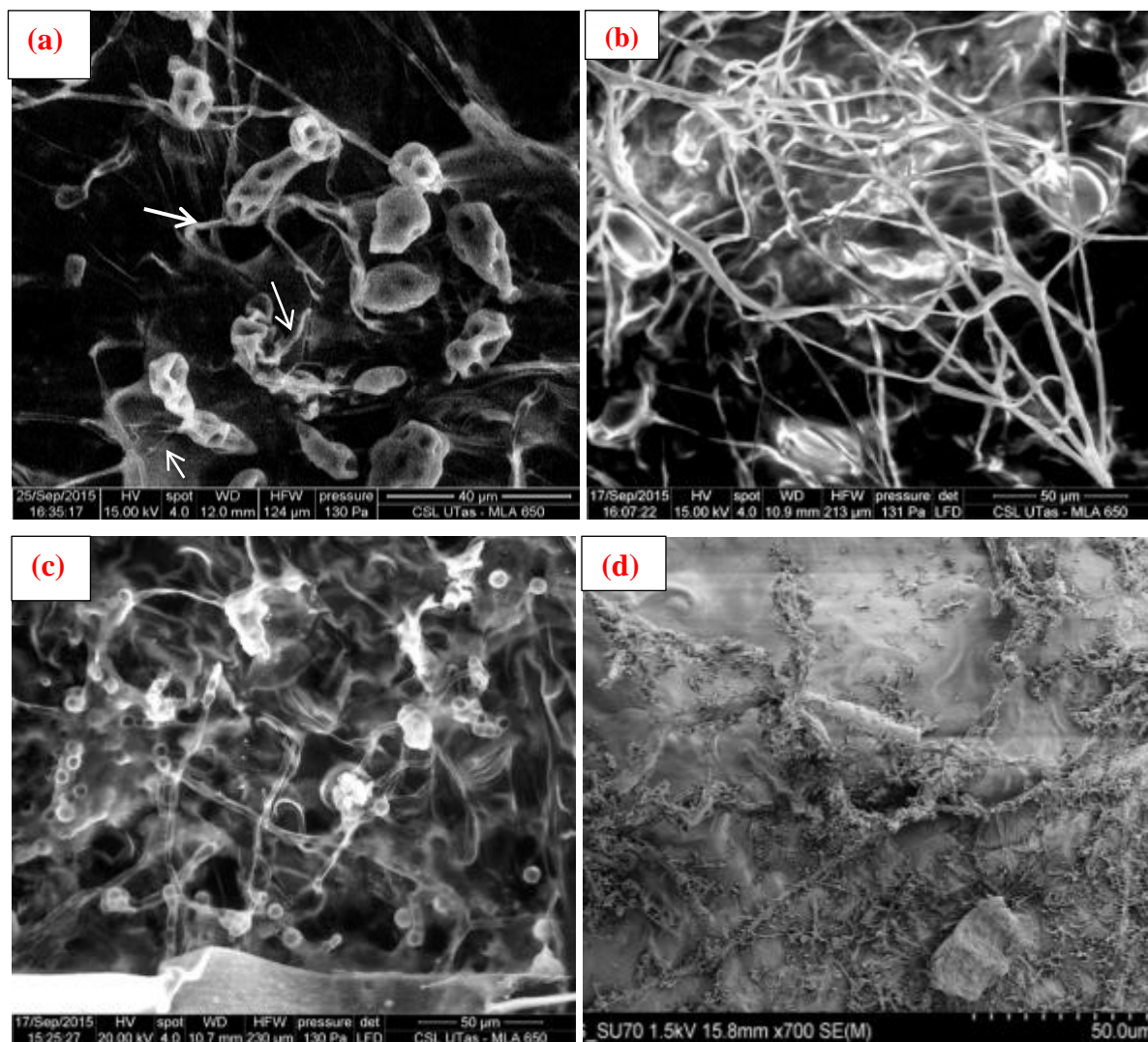
Within the column, values sharing the same letter are not significantly different at  $P = 0.05$ , LSD= 11.65.

#### *Detached leaf assay - visualisation of CCT microbe-pathogen interaction*

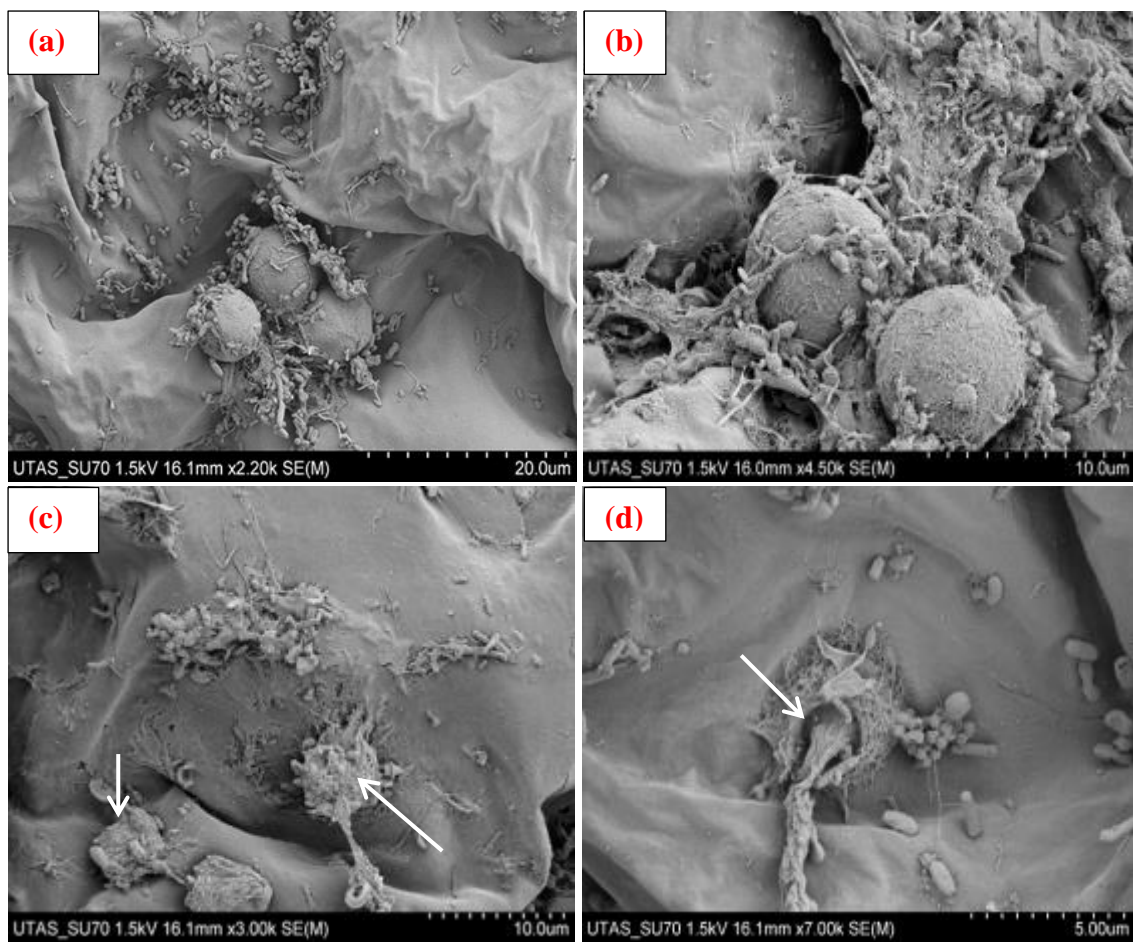
ESEM and SEM images were able to discern fungal and bacterial structures on the leaf surface. The hyphae of *A. alternata* consisted of swollen (2.7  $\mu\text{m}$  – 4.9  $\mu\text{m}$  width) and slender (0.8 $\mu\text{m}$ –1.9  $\mu\text{m}$ ) types, while conidia were multicelled macroconidia (17.2  $\mu\text{m}$  x 8.8  $\mu\text{m}$  – 24.5  $\mu\text{m}$  x 11.1 $\mu\text{m}$ , very short beak (2.4  $\mu\text{m}$  x 1.8  $\mu\text{m}$  – 3.2  $\mu\text{m}$  x 2.8  $\mu\text{m}$ ) to beakless circular structures). In ESEM images of the surface of potato leaves which were inoculated with the pathogen only, hyphal masses (both in slender and swollen types) and conidia (some forming germ tubes) could be seen (Fig. 5a & b). In these control leaves, the conidia had a globous and sunken appearance along with hydrated and extensively growing hyphal masses showing that there was full germination and growth of the pathogen after 7 days of incubation.

Both ESEM and SEM images revealed that for leaves treated with CCT, microbes were evident that were not present in the untreated leaves and that there was visible association between the CCT microbes and conidia and hyphae of the pathogen (Fig. 5-7) on the leaf surface. After incubation of the leaves, microbes from compost teas were shown to adhere to the hyphae and conidial growth structures of *A. alternata* (Fig. 5-7). ESEM images of compost tea treated leaves showed microbes associated with the fungal structures of *A. alternata* (Fig. 5c); however, the extent of the interaction was not very clear and surface details were not crisp even at the higher magnification. In contrast, when observed by SEM, it was clear that an array of NCT microbes were positioned on the hyphae of the pathogen (Fig. 5d).





**Figure 5** ESEM images of fungal (*Alternaria alternata*) growth structures on untreated leaves (a & b) and effect of CCT (commercial compost tea) microbes on the fungal structures (c & d): ESEM of a) Conidiophores of *A. alternata* (arrows: slender germ tubes); b) mycelia of *A. alternata*, c) Growth of compost tea microbes on fungal hyphal masses and conidia & d) SEM image of apparent coverage of *A. alternata* mycelia with NCT microbes. In all cases, detached potato leaves were incubated for 7 days at 25 °C and 60% relative humidity.

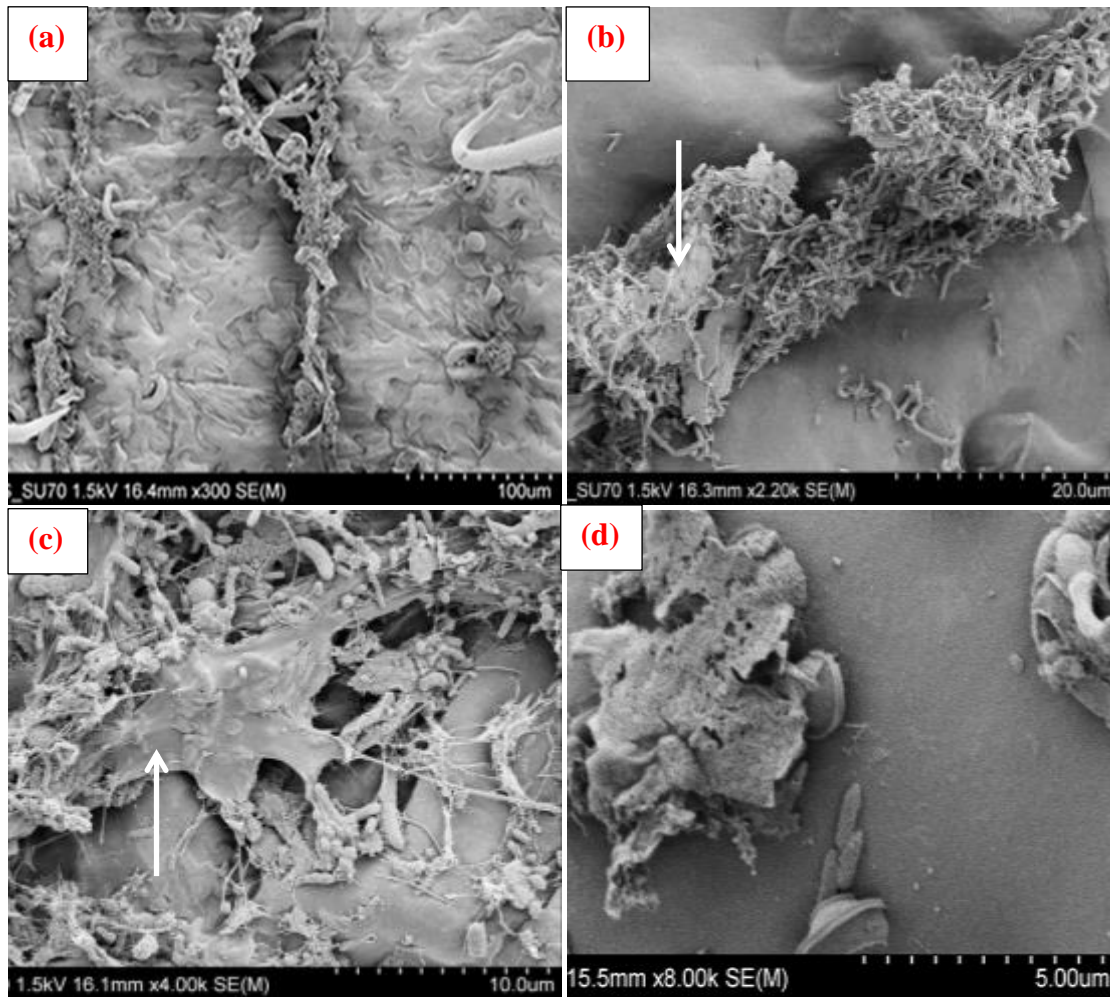


**Figure 6** SEM images of interaction between NCT microbes and pathogen growth structures a) microbes apparently coating *A. alternata* conidia; b) Higher magnification of NCT microbes (bacteria, filamentous fungi) growing in an interwoven fashion along the conidia; c) growth of NCT microbes over the conidia (arrow); and d) apparent rupture and lysis of *A. alternata*. In all cases, detached potato leaves were incubated for 7 days at 25 °C and 60% relative humidity.

The pathogen structures, mainly larger conidia ( $67.1 \mu\text{m} \times 53.2 \mu\text{m}$  –  $74.6 \mu\text{m} \times 57.3 \mu\text{m}$ ), appeared to be coated by microbes, predominantly bacteria (Fig. 6a-d). In SEM images at lower magnification (Fig. 6a), a mixture of cocci-shaped and rod-shaped bacteria were observed on the surface of the conidia. There also appeared to be extensive aggregation and interwoven growth of the bacteria and the fungus with an amorphous structural growth over the conidia apparent at higher magnification (Fig. 6b). Morphological anomalies

including shrinkage and collapse of colonised conidia were observed in the *A. alternata* at the highest SEM magnification (5µm) (Fig. 6c & d).

The addition of a plant-derived gum as an adjuvant to a compost tea appeared to enhance the adherence and attachment of microbes to each other through the formation of coat-like matrix materials. In mycelium inoculated and NCT+G treated leaves, complete attachment and overgrowth of microbes was observed along with the apparent disintegration of the hyphal mass (Fig. 7a). Higher magnification of the interaction of NCT microbes with the pathogen revealed that microbes were cohesively attached to a multilayered structure along the hyphae (Fig. 7b). On most areas of leaf samples treated with NCT + G, microbial morphotypes resembling bacteria and filamentous fungi were seen to form a network with a profuse amorphous intercellular matrix (Fig. 7c & d).



**Figure 7** SEM images of the interaction of NCT microbes and added gum on fungal structures: a) microbe-mycelia interaction at lower magnification b) microbe-mycelia interaction at higher magnifications showing extensive attachment of microbes to each other through a matrix of sheet-like formation (arrow) across the section of mycelium c) spatial distribution and attachment of NCT microbes with an added gum-bacteria, and filamentous fungi attached to each other in extensive masses of the sheet-like matrices (arrow) and d) higher magnification of the extensive sheet-like mass that was distributed on the NCT microbes-pathogen interaction area of the leaf samples. In all cases, detached potato leaves were incubated for 7 days at 25 °C and 60% relative humidity.

### 3.5. Discussion

The results of this study support the importance of the biotic components of compost extracts in suppressing disease caused by soil or foliar plant pathogens (Alfano *et al.*, 2011; Pane *et al.*, 2012). Each NCT that had been filter sterilised did not suppress mycelial growth of the three test pathogens, indicating the key role of living microbes (and probable absence or ineffective quantities of water-extractable antifungal compounds). While the microbial composition and diversity the microbial data represents the community at one time point, this provides an example to compare the NCTs used.

Antagonism, direct or indirect, has been described as a biological control mechanism that refers to a negative outcome when microbes associate with a plant pathogen (Pal and Gardener, 2006). As the ESEM and SEM images show direct contact between what appeared to be bacteria from the CCT and the pathogen structures, direct antagonism might explain the pathogen suppressive effects associated with the microbes present in the CCT tested in this study, through either hyper-parasitism or competition with the pathogen for occupation of the physical niche or for nutrients. While this speculation requires further study, previous studies have shown that the microbial components of compost teas had suppressive effects against various fungal pathogens of horticultural crops including *Alternaria solani*, *Botrytis cinerea*, *Phytophthora infestans*, and *Pyrenochaeta lycopersici* in *in vitro* experiments (Koné *et al.*, 2010; Pane *et al.*, 2012).

It is expected that NCTs from both compost sources (VC and CC) support the growth and proliferation of microorganisms because of the tolerable neutral pH values (Gómez-Brandón *et al.*, 2008) and EC that did not exceed the optimum limit values (range from 3 to 5 dS/m) (Shrestha *et al.*, 2011b; Soumaré *et al.*, 2002). Considering the available total C and total N,

the substrates in the CC were subjected to a more complete decomposition process as compared to the VC. Moreover, high  $\text{NO}_3\text{-N}$  and low  $\text{NH}_4\text{N}$  concentrations of CC were one indication that the compost has been in a curing stage with rather stable microbial activity. Literature indicates that compost with these characters would have a stable microbial activity (Brewer and Sullivan, 2003; Danon *et al.*, 2007; Tiquia *et al.*, 2002). In specific cases where cured compost with high concentrations of  $\text{NO}_3\text{-N}$  was tested against a fungal pathogen, *Sclerotium rolfsii*, the suppressive activity declined as compared to the non-cured samples of the same compost type (Danon *et al.* (2007). These authors attributed the difference in bioactivity to the presence of a uniquely high relative abundance of bacterial phyla in non-cured compost such as Bacteroidetes, Chloroflexi, and different classes of Proteobacteria (Danon *et al.*, 2008). In this study, although the diversity indices were higher in CCT than the VCT, both NCTs had similar community composition and abundance at higher taxonomic levels. The higher suppressive efficacy of the VCT compared to CCT against *A. solani* and *R. solani*-299 in the *in vitro* assay, at all concentrations applied, may be because of the presence of certain minor populations (presumably due to the decomposition stage of the parent compost) whereas the efficacy of the more diverse bacterial and fungal CCT was more concentration dependent.

Application of CCT restricted the development of disease symptoms in the detached leaf assays in wounded and non-wounded leaves compared to the complimentary water controls. In this study, the maturity of leaves used, temperature, lighting, and relative humidity were optimal for pathogen infection as *A. alternata* was sensitive to these factors in previous reports (Pleysier *et al.*, 2006; Reis *et al.*, 2006; Slavov *et al.*, 2004). We found that CCT restricted symptoms on leaves inoculated with mycelium after 7 days of co-



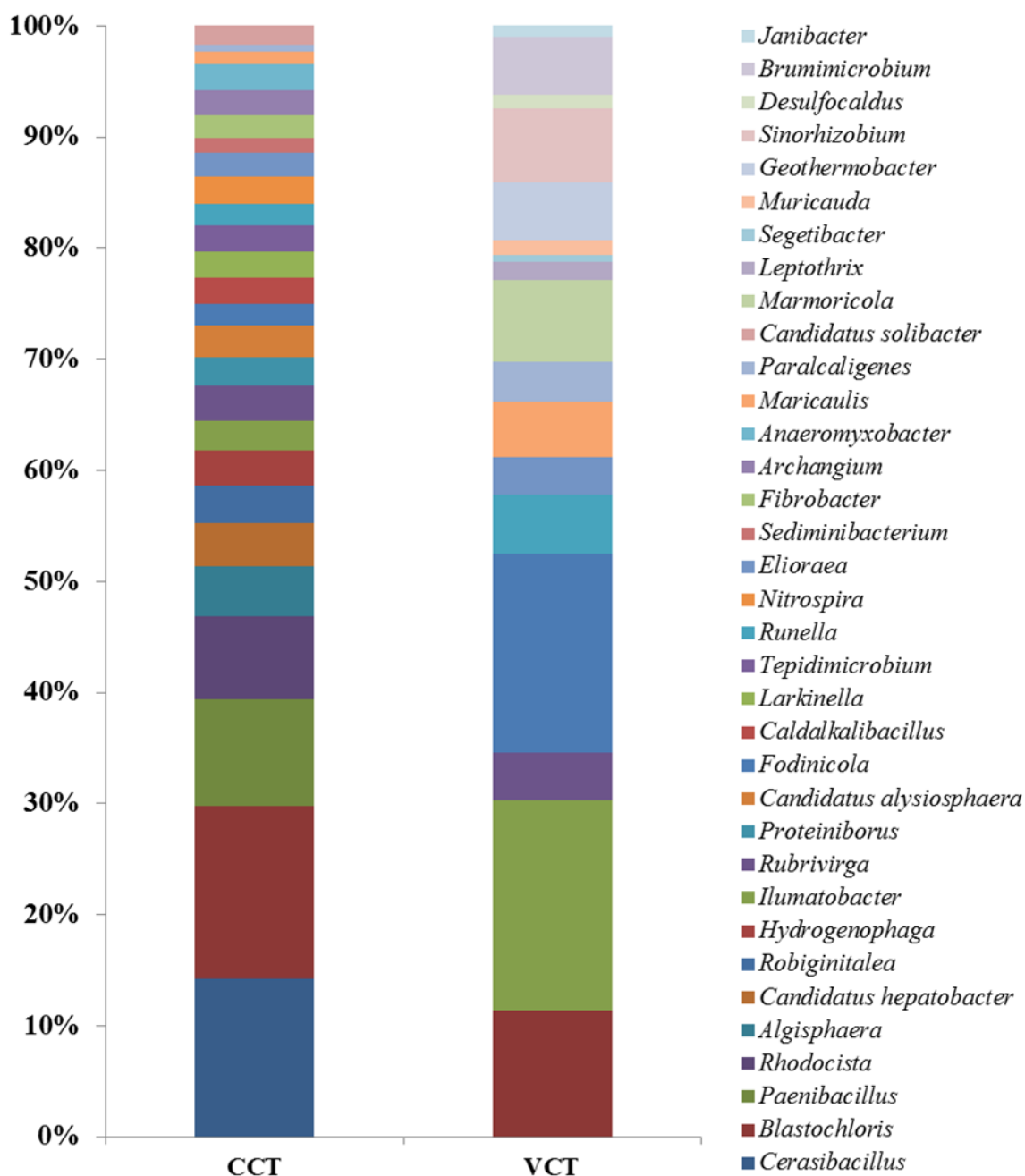
incubation, regardless of whether they were wounded. However, the infection level in water-treated wounded leaves was significantly higher than the unwounded leaves, as wounding most likely facilitated rapid infection of the leaf tissue by the pathogen. Tymon (2014) and Pleysier *et al.* (2006) found that wounding leaves in potato and *Paulownia* trees resulted in the development of larger lesions from *A. alternata*.

Microbial aggregation and biofilm formation, mostly triggered by molecular and cellular mechanisms, is an important strategy for biological control of plant disease (Bais *et al.*, 2004; Zeriouh *et al.*, 2014; Danhorn and Fuqua, 2007). However, certain external factors other than cellular mechanisms are also known to trigger the association and attachment of microbes on the phylloplane. For example, different classes of plant derived natural products such as essential oils (Arrebola *et al.*, 2010) and saponins (Hao *et al.*, 2011), when mixed with strains of *Bacillus amyloliquefaciens*, were reported to increase the biofilm formation and thus suppression of pathogens of peach fruit and citrus mould, respectively. In their work to understand the role of plant polysaccharides as an environmental cue for biofilm formation, Beauregard *et al.* (2013) suggested that plant extracts composed of polysaccharides can be digested by *Bacillus subtilis* to form the external EPS (exopolysaccharides) of the biofilm matrix in colonizing *Arabidopsis thaliana* roots. In our experiment, gum arabic sold as food supplements (FAO/WHO, 1999) contains a mixture of polysaccharides, that could possibly be carbon sources for the diverse NCT microbes. Previous reviews highlighted the role of gum arabic, xanthan gum, and other related biopolymers for stability, colonization and persistence on plant foliar or root surfaces as components of biocontrol formulations (Burges, 2012; Dey *et al.*, 2014; Junaid *et al.*, 2013; Schisler *et al.*, 2004).

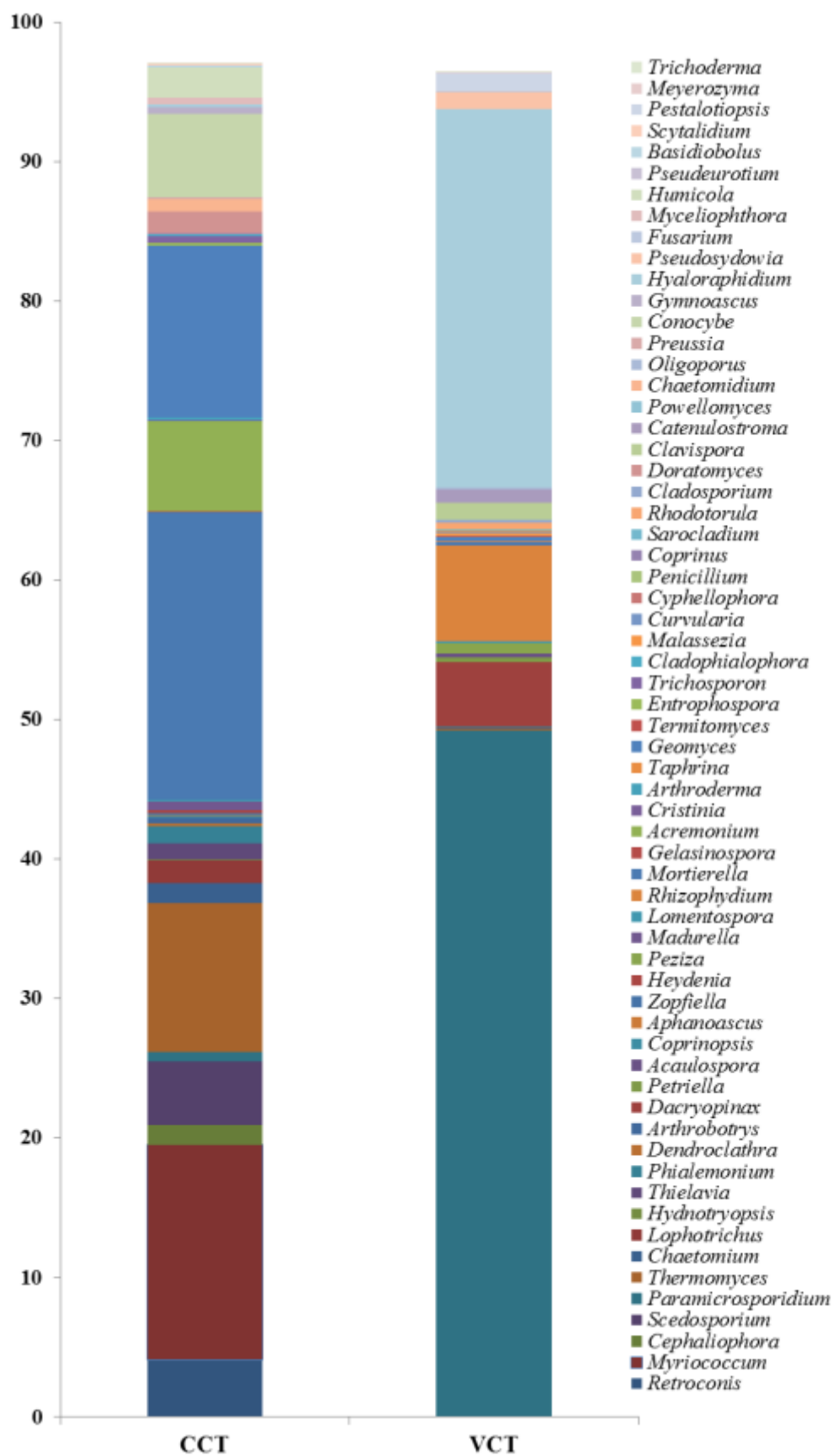
While the presence of gum did not alter the efficacy of the NCT in this study, an enhanced degree of microbial attachment was observed and appeared to be associated with the presence of polysaccharides. The gum may have contributed to exopolysaccharide synthesis and thus to the appearance of biofilm-like structures (Beauregard *et al.*, 2013). The extent of biofilm formation was not investigated in our study; however, the micrographs revealed that the carbohydrate gum addition had markedly altered the leaf surface. Large masses of amorphous intercellular matrix, peculiar to biofilm architecture, were seen on leaves treated with CCT+Gum. Further research, including *in situ* trials, is required to evaluate the addition of polysaccharide rich adjuvants such as gum arabic to compost tea to improve the consistency of disease suppression by this form of biological control. The effect of compost tea with gum on leaf surfaces in terms of physiology, photosynthesis, and their effect on stomata are relevant factors that need investigation.

In summary, the parent composts contained microbial taxa that inhibited the *in vitro* growth of the tested fungal mycelia. CCT harbouring diverse microorganisms also suppressed *A. alternata* leaf spot on detached leaves, with the mechanism postulated to be hyper-parasitism and/or competition. Adding gum as an adjuvant appeared to enhance the aggregation of the microbes on the leaf surface; however, further research is needed to evaluate if such aggregation of microbes from CCT could enhance leaf competence and successful colonization of the leaf surface.





**Figure S1** Relative abundances expressed as percentage of the total number of sequences of the major bacterial genera in both compost teas: CCT (commercial compost tea) & VCT (vineyard compost tea)



**Figure S2** Relative abundances expressed as percentage of the total number of sequences of the major fungal genera in both compost teas: CCT (commercial compost tea) & VCT (vineyard compost tea)

# Chapter 4

## DIVERSE MICROBIAL COMMUNITIES IN NON-AERATED COMPOST TEAS SUPPRESS BACTERIAL WILT

### 4.1. Abstract

Non-aerated compost teas (NCTs) are water extracts of composted organic materials and are used to suppress soil borne and foliar disease in many pathosystems. Greenhouse trials were used to test the effectiveness of NCTs to suppress potato bacterial wilt caused by *Ralstonia solanacearum* on plants grown in soils inoculated with a virulent isolate of the pathogen (biovar II). NCTs prepared from matured compost sources: agricultural waste (AWCT), vermicompost (VCT) and solid municipal waste (SMWCT) were evaluated at three initial application times (7 days before inoculation, at time of inoculation and 7 days after inoculation) prior to weekly applications, in a randomized complete-block design. AWCT applied initially at the time of inoculation resulted in the greatest disease suppression, with the disease severity index 2.5-fold less than the non-treated plants and the “area under the disease progress curve” (AUDPC) 3.2-fold less. VCT and SMWCT were less suppressive than AWCT regardless of initial application time. Next generation sequencing of the v4 region of 16S rRNA gene and the internal transcribed spacer region (ITS1) revealed that diversity and composition of the bacterial and fungal communities across the NCTs varied significantly. Dominant bacterial phyla such as *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, and a fungal phylum *Ascomycota* were detected in all NCTs. AWCT had optimum physico-chemical measurements with higher bacterial Shannon diversity indices (H) and

fungal richness (S) than the other treatments. We conclude that bacterial wilt of potatoes grown in controlled-environment conditions can be suppressed by a non-aerated compost tea with a high microbial diversity when applied at planting and weekly thereafter.

## 4.2. Introduction

Bacterial wilt, caused by *Ralstonia solanacearum* (Álvarez et al., 2010b; Yabuuchi et al., 1995), is regarded as one of the most severe diseases of potato, causing great economic loss to production worldwide (Ding et al., 2013). Mostly found in tropical and subtropical regions, the bacterium is known to affect more than 200 plant species distributed among 50 botanical families (Hayward, 1991). Various strains of *R. solanacearum* have been associated with the loss in yield and quality of important crops such as tomato, eggplant, pepper, tobacco, banana, chili, and peanut (Álvarez et al., 2010b; Kempe and Sequeira, 1983). Control strategies for a range of hosts have been developed; these include synthetic chemical pesticides (Lee et al., 2012), cultural practices such as field sanitation, clean seed production and crop rotation (Kassa and Chindi, 2013), induced resistance by natural products and elemental nutrients such as silicon and calcium treatments (Gado, 2013; Dannon and Wydra, 2004), and non-pesticide chemicals such as Acibenzolar-S-methyl (ASM) (Pradhanang et al., 2005). The use of pesticides as fumigants or disinfectants is not only associated with environmental contamination and human health risks (Acero et al., 2008), but also with depletion of beneficial soil microbes associated with the suppression of the pathogen population (Gamliel et al., 2000). Similarly, resistance inducing chemicals options are less practical because they are more expensive than other options (Yuliar and Toyota, 2015).

Biologically based treatments particular to specific hosts have shown promising results as crop protectants in different experimental settings. Amendment of soil organic content by incorporating composted animal and crop residues (Li and Dong, 2013), and a range of isolated rhizosphere and endophytic beneficial microbes (Tan *et al.*, 2013) have been reported as effective and environmentally friendly crop protectants. Populations of *R. solanacearum* are genetically variable (Álvarez *et al.*, 2010b) and capable of adapting to a range of environmental conditions (van Elsas *et al.*, 2000) thus making biological control less effective. Bacterial wilt control is generally an ongoing challenge for farmers and a universally accepted management option for many plant hosts is lacking.

Integrated management of bacterial wilt, by incorporation in the soil of a range of solid organic matters such as compost, slurries of animal wastes, bio-organic fertilizers, as well as their extracts, provide a promising tool that primarily increase microbial antagonism against *R. solanacearum*. Bioorganic fertilizer derived from a mixture of animal and plant-based organic products mixed in water without subsequent fermentation was found to suppress bacterial wilt development in certain hosts, such as tobacco (Wu *et al.*, 2014) and potato (Ding *et al.*, 2013).

The suitability of water extracts of composted organic materials (“compost teas”) in suppressing various diseases of a wide range of horticultural crops has been studied extensively (Evans and Percy, 2014). Effectiveness of compost tea against range of soil borne fungal pathogens such as *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* has been reported in various horticultural crops (Pane *et al.*, 2014). There are few reports that have indicated the suitability of compost tea for suppression of bacterial disease. For example, compost teas of different sources have significantly reduced the severity of tomato foliar spot caused by *Xanthomonas vesicatoria* (Al-Dahmani *et al.*, 2003). Similarly, Islam *et al.* (2014) reported that a soil drench of compost tea suppressed the severity of bacterial wilt in brinjal, caused by *R. solanacearum*. There do not appear to

be any reports of bacterial and fungal diversities from NCTs used for suppressing bacterial wilt of potato caused by *R. solanacearum*.

The suppressiveness of compost tea is mostly ascribed to its biotic component (Koné *et al.*, 2010). Previous studies suggested that total numbers of culturable microorganisms (microbial abundance within the range studied) was not associated with the degree of disease suppression (Pane *et al.*, 2012), whereas microbial community structure appeared to play a role in the suppression of gray mold on geranium after foliar application of compost tea (Scheuerell and Mahaffee, 2006). Palmer *et al.* (2010) also reported an association between the diversity of culturable bacterial and fungal microbes and disease suppressive ACTs. Therefore, understanding the microbial community structure in compost tea is likely to be important in optimising production protocols. Microbial community composition and diversity in compost tea have been mostly studied by culture based techniques (Koné *et al.*, 2010), or culture independent approaches using fingerprinting techniques (Shrestha *et al.*, 2011a), that provide limited taxonomic resolutions. Application of next generation sequencing technologies provides a powerful tool to source information about microbial community structure by extracting community DNA and sequencing the phylogenetic gene targets (16S r RNA, bacteria) and ITS regions for fungi (Caporaso *et al.*, 2012; Lindahl *et al.*, 2013) from environmental samples. Therefore, this study was designed to evaluate the potential of non-aerated compost teas produced from variable compost sources in suppressing bacterial wilt of potato. Microbial communities of the NCTs were studied to determine if variation in diversity, abundance, and richness of both bacterial and fungal microbes at different taxonomic levels were related to efficacies of compost teas applications.

### 4.3. Materials and Methods

#### *Composting conditions and feedstock composition*

Agricultural waste compost (AWC) and vermicompost (VC) were prepared in an experimental field of the Ambo Plant Protection Research Centre, located at 8°57'N, 37°52'E, Ethiopia between April to June, 2013.

Briefly, various types of agricultural waste, composed of plant and animal based materials (maize and wheat straw, chopped grasses, wood ash, cow dung, and forest soils), were piled in equal proportion. The composting process was initiated with wetting of the piles by agricultural well water and then the piles were allowed to decompose for 65 days in a pit composting system (size approx. 1 cubic m), which is a typical composting technique in the area. The compost was left for further curing and maturation for 5 months until it was used for the trials. In vermicomposting, earthworms (*Eisenia fetida*) were used to decompose the starting organic waste substrates. The bedding, consisting of animal waste (donkey manure), and plant based waste (wheat straw and corn stalks) were mixed in equal amounts in plastic composting boxes, with holes drilled in the bottom for aeration and drainage, according to the local practice. Solid municipal waste compost (SMWC) was made by the Addis Ababa Environmental Protection Authority. Compostable and sorted household solids consisting of vegetable and fruit peelings and other food wastes were collected from residential houses near the site and composted in an open windrow method for a period of 3 months and further cured for 3 months.

#### *Preparation of compost tea*

Matured samples of AWC, VC, and SMWC were used for preparation of non-aerated compost teas (NCTs) according to the procedure outlined by Koné (2010). Plastic buckets (30 L capacity) were used



to prepare the different batches of NCTs in 1:5 ratios (w/v) using agricultural well water for a period of 14 days at room temperature. Layered muslin cloth was used to filter the respective compost ferments. For use in disease evaluation trail, freshly prepared compost tea batches were used. For both physico-chemical and microbiological analysis, samples were stored in a cool room (5–8 °C) for a period four days until transferred to analytical laboratories.

#### *Isolation and culture of Ralstonia solanacearum*

*R. solanacearum* used in this trial was isolated from a local farmer's tomato (*Solanum lycopersicum* L.) field near Ambo, Ethiopia. As survey of emerging potato plants in adjacent farms did not show wilt incidence, samples from tomato plants showing typical symptoms of bacterial wilt were considered and brought to the laboratory for isolation, identification and maintenance of the pathogen. Samples from stems and roots of the plants were surface sterilized with 1% sodium hypochlorite for 3 minutes, cut into pieces and separately transferred to a selective 2, 3, 5-triphenyltetrazolium chloride agar (TZC) medium (Kelman, 1954). After 48 h, 11 mucoid, reddish and irregularly shaped colonies having a central white colour typical of wild/virulent types of *R. solanacearum* were purified by selecting individual colonies and subculturing onto the same media (Kelman, 1954). A hypersensitivity test was conducted; single colonies of the isolates from the 48 h old culture were transferred to 250 ml of liquid nutrient broth and grown for 48 h on a rotary shaker at 150 rpm at room temperature. A 3 ml volume from the stock bacterial solution (adjusted to  $10^9$  cfu) of each isolate was intravenously injected into the leaves of tobacco (*Nicotiana tabacum*) and the leaf reaction observed for 24 to 72 h (Kempe and Sequeira, 1983). Based on cultural characteristics and hypersensitivity response, three isolates were selected and maintained in sterile distilled water at room temperature for further biochemical tests and soil inoculation in the greenhouse experiments.

### *Biochemical characteristics of Ralstonia solanacearum isolates*

Selected biochemical characterization of three chosen isolates, including Gram staining, KOH solubility test, oxidase test, catalase test and starch solubility test were performed according to standard procedures (Goszczyńska *et al.*, 2000). For the biovar identification, carbon source utilization tests were conducted based on the ability of each isolate to utilize disaccharides (sucrose, lactose and maltose) and sugar alcohols (mannitol, sorbitol and dulcitol) according to the procedure described by Aley and Elphinstone (1995) using Hayward's basal medium (Hayward, 1964). The carbohydrates were prepared in 10 % w/v water, sterilised and transferred to the previously autoclaved basal medium in test tubes. Loops full of 48 h old culture inoculum of each of the three isolates were prepared in 300 µl of sterile water to make individual bacterial suspensions. A volume of 30 µl of each isolate suspension was added to basal media amended with the carbohydrate sources, incubated for up to 3 weeks and then observed at 3, 7, and 14 days for the formation of top to downward yellow coloration due to the change in pH (Aley and Elphinstone, 1995).

### *Characterisation of compost and NCTs*

Physicochemical and microbial analysis of compost and NCTs:

Physical and chemical parameters such as electrical conductivity (EC), pH (1: 2.5 H<sub>2</sub>O) and extractable chemical nutrients (Na, K, Ca, Mg, Fe, Mn, Cu, and Zn) were analysed according to standard procedures for both the compost and nonaerated compost teas at the Soil Testing Laboratory of Ethiopia. Quality parameters including organic carbon content (OM) and total nitrogen (TN) were measured for the parent composts at the start of the trial to quantify the C: N ratio of the matured compost used for production of the NCTs.

Microbial community analysis:

In order to characterise the microbial communities, genomic DNA was extracted from the last batch of each NCTs used for the disease trial, using the Power Soil DNA kit (MO BIO Laboratories, Inc., USA) according to the manufacturer's instruction. A 0.25 g pellet collected from centrifugation of 30 ml of NCT samples at a speed of 2900g for 30 min was added to bead-beating tubes and further purified through the subsequent steps of the extraction procedure. DNA was extracted in triplicate from each NCT sample and purity was measured using the spectrophotometer at wavelengths of 260/280 nm (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE, U.S.A.). To determine the diversity and composition of the bacterial and fungal communities in the non-aerated compost teas, high throughput amplicon sequencing was conducted according to the methods described by Caporaso *et al.* (2011).

Microbial genes sequencing of NCT samples was performed at MR DNA (Shallowater, TX, USA) on the Illumina MiSeq platform following the manufacturer's guidelines. From DNA samples of the NCTs, the V4 variable region of the 16S rRNA gene (for bacteria and archaea) was amplified with the PCR primers 515F/806R and the internal transcribed spacer region (ITS1) of the nuclear ribosomal RNA gene (for fungi) was amplified with primers ITS1-F/ITS2. Amplifications were performed in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). The PCR conditions were: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. PCR products were checked by gel electrophoresis using a 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple PCR product samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR products were used to prepare a DNA library by following the Illumina TruSeq DNA library preparation protocol. Sequence data were processed using the MR DNA analysis pipeline (MR DNA,

Shallowater, TX, USA). Briefly, sequences were joined, depleted of barcodes, denoised, then ambiguous base calls, chimeras and sequences with length of <150bp were removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes (DeSantis *et al.*, 2006), RDP11 and NCBI ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov), <http://rdp.cme.msu.edu>). Sequence data from the project have been deposited in the MG-RAST (metagenomics analysis server) under accession numbers for bacteria: MGP15434, and for fungi: MGP15436.

### *Statistical analysis*

PRIMER 6 (version 6.13) & PERMANOVA+ (version 1.0.3) software packages (PRIMER-E, Plymouth, Ivybridge, United Kingdom) were used for the assessment of microbial community composition of the samples. Bray Curtis similarity coefficients were calculated between pairs of samples based on untransformed percentages of the operational taxonomic units (OTUs) and created as a lower triangular resemblance matrix. Permutational multivariate analysis of variance (PERMANOVA) was used to assess the effect of non-aerated compost tea type on observed bacterial and fungal composition. Principal coordinate analysis by Canonical Analysis of Principal Coordinates (CAP) was used to make the ordination diagram showing the structural difference between communities (Anderson and Willis, 2003). The relative proportions of bacterial and fungal taxa, at phylum, class and genus levels, were calculated and used to construct a table illustrating the variation in relative abundance of both bacterial and fungal microbes in the replicates (n=3) of the compost teas samples. Alpha diversities (species richness S and Shannon diversity index H) of both fungal and bacterial communities were determined based on the number of OTUs observed.

### *Greenhouse experiment*

Plant material, inoculum and inoculation method:

Sprouted tubers of potato (*Solanum tuberosum* L.) of the variety *Jalene* were obtained from the seed multiplication section of Holetta agricultural research laboratories. Single tubers were planted in pots (20 cm diameter) which were filled with a mixture of soil prepared from field soil, compost and sand in 2:1:1 ratio, respectively. The soil mixture was autoclaved at 121°C for 2 h and cooled before tubers were planted. Each planted tuber gave rise to three to five main stems.

For the soil inoculation, a pathogenic isolate of *R. solanacearum* was grown on TZC medium for 48 h. It was then transferred for mass production in a nutrient broth for an additional 48 h at a room temperature. Pots allocated for inoculation with *R. solanacearum* were drenched with 200 ml bacterial suspension of  $10^9$  cfu/ml. Inoculations occurred 7 days before or after sprouted tubers were transplanted to the pots according to the treatment, as outlined below. Control pots were drenched with 200 ml of distilled water. The temperature of the greenhouse was maintained in the range 25–32°C and relative humidity was approximately 70% for the experimental period of 80 days.

Treatments:

NCTs extracted from the three compost sources above were evaluated for their ability to suppress bacterial wilt development using the potted potato plants. A randomised complete block design with three blocks (replicates) was used. Within each block, there were 10 treatment combinations comprising compost tea type and application timing (Table 1). Each compost tea was either applied as a 500 ml drench 7 days before soil inoculation with *R. solanacearum* (AE, SE , VE) at the same time as the inoculum (AO, SO,VO) or 7 days after pathogen inoculation (AI, SI, VI ). Each treated plant then received a foliar spray of 500 ml of the designated compost tea on a weekly basis throughout the remaining experimental period. The control treatment plants were inoculated and non-treated; rather, distilled water was applied on each of the days a compost tea treatment was applied (Table 1).

**Table 1.** List of treatments used to evaluate the suppression of potato bacterial wilt in the greenhouse experiment

Treatment Code	Compost tea type	Application time
<b>AI</b>	Agricultural waste (AWCT)	7 days after inoculation
<b>AO</b>	Agricultural waste (AWCT)	At inoculation
<b>AE</b>	Agricultural waste (AWCT)	7 days before inoculation
<b>SI</b>	Solid Municipal waste (SMWCT)	7 days after inoculation
<b>SO</b>	Solid Municipal waste (SMWCT)	At inoculation
<b>SE</b>	Solid Municipal waste (SMWCT)	7 days before inoculation
<b>VI</b>	Vermicompost tea (VCT)	7 days after inoculation
<b>VO</b>	Vermicompost tea (VCT)	At inoculation
<b>VE</b>	Vermicompost tea (VCT)	7 days before inoculation
<b>CI</b>	Inoculated control (C)	No compost tea applied

Disease assessment:

Yellowing and stunting of the aboveground plant parts, typical of the symptoms of bacterial wilt, were first observed 1 month after soil inoculation. Nine plants per treatment were considered for disease scoring. Disease severity was assessed for each potted plant at 32, 42, 52, 62 and 72 days after planting using a 0–4 scale (Kempe and Sequeira, 1983), where 0 = no symptoms, 1 = up to 25 % of the foliage wilted, 2 = 25–50% of the foliage wilted, 3 = 50–75% of the foliage wilted and 4 = 75–100% of the foliage wilted.

Data analyses:

The mean disease rating was calculated from the severity scores for each of the three replicates per treatment. This mean was then expressed as a percentage of the maximum possible score (4) to express disease severity on a scale of 0–100 (Winstead and Kelman, 1952). Disease severity index (DSI)

(%) assessed at the different times after planting was used to calculate the area under the disease progress curve (AUDPC) by the method of Campbell and Madden (1990) as:

$$\sum_{i=1}^{n-1} \left( \frac{x_{i+1} + x_i}{2} \right) (t_{i+1} - t_i)$$

Where  $X_i$  is the disease index expressed as a proportion at the  $i^{\text{th}}$  observation;  $t$  is the time (days after planting) at the  $i^{\text{th}}$  observations; and  $n$  is the total number of observations

The presence or absence of treatment effects was tested using one way analysis of variance (ANOVA) with the GLM procedure for the final disease severity index and AUDPC response variables using the SAS software program (SAS Institute, 2011). When effects of treatments were significant ( $P < 0.05$ ), means were compared using Fisher's protected least significant difference (LSD) test.

#### 4.4. Results

##### *Characteristics of Ralstonia solanacearum*

Eleven isolates were screened for pathogenicity and hypersensitivity tests. Of these, three isolates of *R. solanacearum* from symptomatic tomato plants showed cultural characteristics typical of virulent strains. These isolates formed highly opaque and smooth colonies on the nutrient agar medium (NA) and produced fluid, brown/tan pigment with whitish-pink centres on TZC medium after 48 h of incubation at 28 °C, which is similar to the documented morphology of *Ralstonia solanacearum* (Kelman, 1954). They were all gram negative, rod-shaped and non-spore forming isolates. They induced chlorotic symptoms on injected tobacco leaves within 3 days, however were not pathogenic to tobacco. The isolates showed positive results when tested for oxidization of maltose and lactose typical of biovar II, unlike the strains of the biovar I which do not produce acid because the carbohydrates supplied are not utilized. The three isolates considered were all positive to oxidase,

catalase and KOH tests and were pathogenic to potato and hence we randomly chose one of them for the subsequent activities. Earlier reports indicated strains isolated from solanaceous vegetables (mainly tomato and potato) in Ethiopia were mainly classified as biovar II, with recent records of biovar I strains from the same host plants (Lemessa and Zeller, 2007; Yaynu, 1989).

#### *Physicochemical characteristics of parent compost and compost teas*

The parent compost sources had pH values ranging from 7.1 to 8.5 and the C: N ratio of the AWC (19.8) was higher than that of the SMC and VC (3.1 and 3.7, respectively). The EC and concentration of extractable cations in the AWC were lower than the values recorded for the SMWC and VC, and this difference was also reflected in the EC for the respective compost teas.

The pH of the parent composts and compost teas was neutral with the SMC being slightly alkaline (pH 8.5) (Table 2). Values for the concentration of available cations and anions were variable among parent composts and compost teas. AWCT appeared to have a lower concentration of the extractable ions K, Ca, Fe, Cu and Zn (Table 2)

**Table 2** Physico-chemical properties of composts and respective compost teas used in the greenhouse study of bacterial wilt suppression.

	pH(1:2.5)	EC (mS/cm)	Na	K	Mg	Ca	Fe	Mn	Cu	Zn	OC (%)	TN (%)	C:N
			Cmol(+)/Kg				ppm						
Parent Composts <sup>a</sup>													
AWC	7.1	1.1	255	1900	3762	629	14.8	76.1	1.8	11.3	9.3	0.5	19.8
SMWC	8.5	19.8	2700	21050	3892	683	67.1	105.5	9.4	68.0	8.0	2.6	3.1
VC	7.4	11.6	1700	17350	3101	801	65.8	108	3.1	37.8	8.5	2.3	3.7
			(ppm)				(ppm)						
Compost Tea <sup>b</sup>													
AWCT	7.5	0.6	145	290	4.5	20.7	6.7	13.6	0.1	0.1	-	-	
SMWCT	7.8	10.3	145	9700	4.7	48.2	27.2	13.4	6.4	5.3	-	-	
VCT	7.8	8.6	125	7850	5.9	71.9	18.8	11.9	4.9	2.3	-	-	

<sup>a</sup> Parent composts: AWC—agricultural waste composed of plant and animal based materials (maize and wheat straw, chopped grasses, wood ash, cow dung, and forest soils) piled in equal proportion by underground pit composting system, SMWC —solid municipal waste compost made from sorted household solids consisting of vegetable and fruit peelings and



food wastes and composted in an open windrow method, and VC— vermicompost made from agricultural waste bedding materials (donkey manure, and plant based waste including wheat straw and corn stalks)

<sup>b</sup> Non-aerated compost teas: AWCT-agricultural waste tea, SMWCT-solid municipal waste tea, VCT-vermicompost teas. The three compost teas were extracted in a ratio of 1:5 (w/v) by agricultural well water for a period of 14 days.

### *Effect of NCT on bacterial wilt disease*

Individual treatments showed variability in disease suppression on each assessment date, as reflected by the values for the final mean disease severity index and AUDPC (Table 3). All NCT treatments, except treatments SI and VI, had lower mean DSIs and AUPDC values than the non-treated control.

AWCT applied concurrently with the pathogen (AO) resulted in a mean DSI of 33%, which was significantly lower than seven days before pathogen inoculation (AE, 45%), seven days after pathogen inoculation (AI, 50%) and the non-treated control (CI, 83.3%) (Table 3). All treatments resulted in lower AUDPC than the control treatment; the AO treatment resulted in the lowest AUDPC of all treatments, followed by the same type of compost tea applied as a protective treatment (AE).

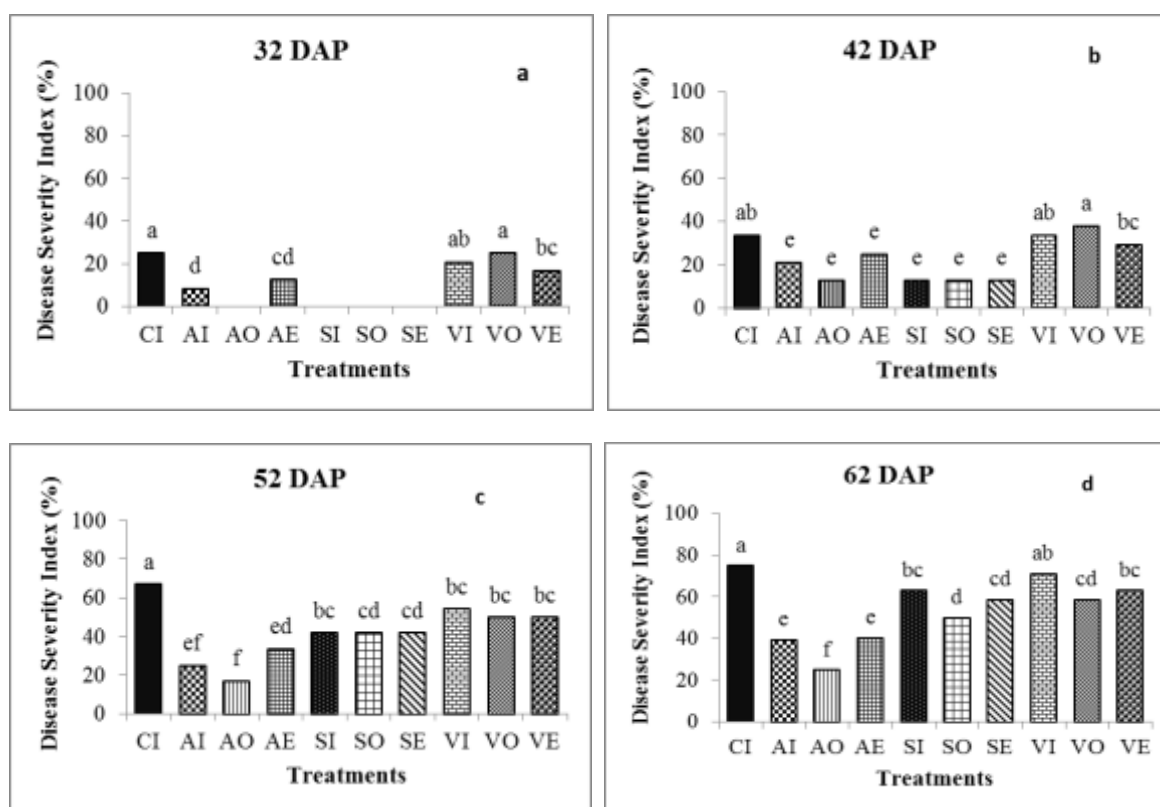
**Table 3.** The effect of compost tea treatment (Table 1) on the severity of bacterial wilt on potato variety *Jalene* grown in pots. Within each column, values sharing the same letter are not significantly different at  $P = 0.05$ .

Treatment	Mean DSI <sup>a</sup> (%) at		AUDPC (%-days) <sup>b</sup>	
	72 days after	planting		
AI	50.0	d	1139	h
AO	33.3	e	708	j
AE	45.8	d	875	i
SI	75.0	bac	1542	ef
SO	54.2	d	1313	g
SE	66.6	c	1458	gf
VI	79.2	ba	2083	b
VE	70.8	bc	1833	cd
VO	66.7	c	1938	bc
CI	83.3	a	2292	a

<sup>a</sup>  $DSI = S_{nr} \times 100 / N_{pr} \times M_{sc}$ ; where  $S_{nr}$  is the sum of numerical ratings of disease severity (0 to 4 scale),  $N_{pr}$  is the number of plants rated and  $M_{sc}$  is the maximum possible numerical rating (4). Treatments sharing the same letter within a column are not significantly different according to Fisher's LSD and  $\alpha=0.05$

<sup>b</sup> AUDPC is the area under the disease progress curve for each treatment; that is, mean DSI (%) against the date of disease assessment. Treatments sharing the same letter across column are not significantly different according to Fisher's LSD and  $\alpha=0.05$

Effects of the weekly application of the treatments on symptom progression were also evaluated at four assessment points (day 32, 42, 52 and 62 after planting) prior to the final disease assessment (Fig. 1 A-D). No disease was detected in any of the plants treated with SMWCT until 42 days after planting. Approximately 50 days after planting, there appeared to be a steep increase in the severity of wilting of plants treated with SMWCT, although the mean DSI and AUDPC remained significantly lower than the control and VCT treatments 72 days after planting (Table 3). AWCT appeared to restrict symptom development consistently across all disease scoring dates compared to the other tea types. Plants treated with vermicompost teas (VI, VO, and VE) showed progressive wilting with time and disease severity indices were often as high as the control treatment at each assessment date. By 52 days, the severity of wilting for plants receiving SMWCTs in all the three application timings (SO, SE, and SI) was similar to the severity observed in the non-treated plants.



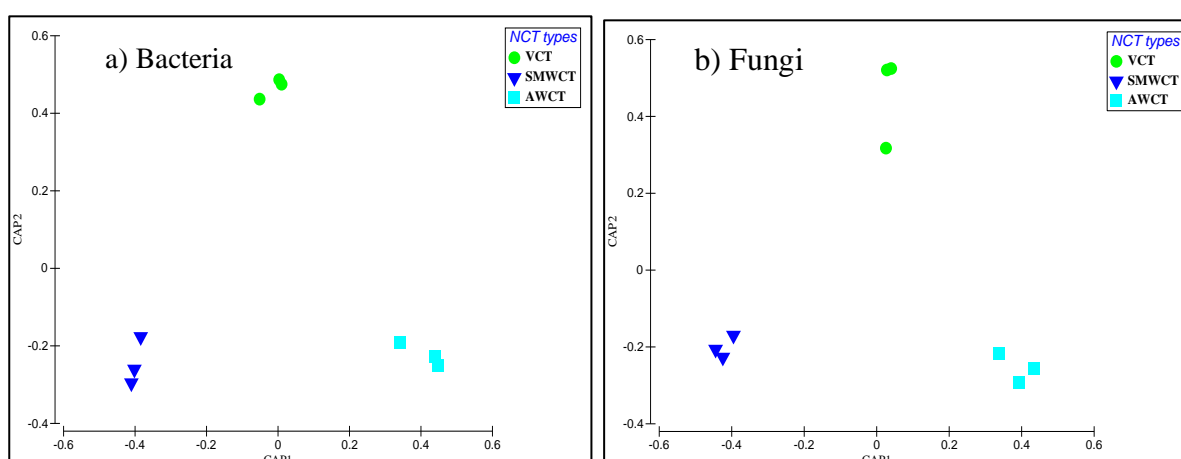
**Fig.1 (a-d)** Mean bacterial wilt severity (%) at different times after planting (A-D) of potato variety *Jalene*. The soil of potted plants, except non-inoculated controls, was drenched with 200 ml of  $1 \times 10^9$  cfu/ml pathogenic *Ralstonia solanacearum*. A 500 ml volume of each non-aerated compost tea of types A, S or V (Table 1) was applied initially at I, O or E (Table 1) and weekly thereafter. Mean ( $n=3$ ) bars sharing the same letter within each sub-figure (a-d) are not significantly different according to Fisher's LSD and  $\alpha=0.05$ .

#### *Microbial community structure and diversity*

A total of 477,335 and 201,374 16S rRNA and fungal ITS rRNA gene sequences were obtained by amplicon sequencing after they had been filtered to remove poor quality reads. Among replicates of the NCT samples ( $n=3$ ), the number of bacterial gene sequences varied from 27, 625 to 70,712

(median=46,606), whereas the number of the fungal ITS gene sequences varied from 6,628 to 36,956 (median =24,070).

The community structure varied significantly with compost tea source. Permutational multivariate analysis of variance showed bacterial and archaeal communities harboured in the NCTs were significantly different ( $p=0.004$  and  $p=0.003$ , respectively). Fungal communities among NCTs were also significantly different ( $p=0.003$ ). Similarly, canonical analysis of principal coordinates (CAP) also showed that both the bacterial and fungal communities in the different NCT types were significantly different ( $p=0.003$  for both analyses) (Fig. 2 A and B).



**Fig. 2 (a-b)** Canonical analysis of principal coordinates (CAP) for A) bacterial, and B) fungal communities in three types of non-aerated compost tea: circles represent vermicompost tea (VCT), triangles represent solid municipal waste compost teas (SMWCT) and squares indicate agricultural waste compost teas (AWCT).

The three NCTs were found to contain very diverse bacterial communities at all levels of taxonomic classification. The main bacterial phyla included *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, and *Acidobacteria* (Table 4).

*Proteobacteria*, mainly *Betaproteobacteria*, was the most common phylum in the AWCT whereas the *Bacteroidetes* was the most common phylum in VCT and SMWCT. The class *Bacteroidia* dominated in SMWCT whereas *Flavobacteriia* dominated the VCT community.

Ascomycota was the dominant fungal phylum in all NCT types (Table 5). *Eurotiomycetes*, *Leotiomycetes* and *Sordariomycetes* were the dominant fungal classes of the phylum *Ascomycota* in all NCTs. SMWCT harboured species of the phylum *Basidiomycota* in higher abundance compared to the other two compost teas (Table 5). AWCT contained a significant proportion of *Ascomycota* (30.65 % relative abundance) for which the class could not be determined, while the presence of unidentified classes was low in the other two compost teas (relative abundance of < 0.5%) (Table 5). Alpha diversity measures varied among NCTs for both the bacterial and fungal communities (Table 6). The bacterial communities in the VCT had the highest richness but lowest Shannon diversity. The fungal communities in the AWCT had the highest richness but Shannon's diversity did not vary greatly between the three compost teas. For the fungal communities, although DNA extraction and analysis were conducted at the same condition, the three SMWCT samples have appeared to show wide variation in ITS diversity ( $H'$ ) (Table 6).

**Table 4.** The mean of the relative abundances expressed as percentage of the total number of sequences of the major bacterial taxa in vermicompost tea (VCT), solid municipal waste compost tea (SMWCT) and agricultural waste compost tea (AWCT). Cells shaded red indicate the highest values for relative abundance.

Phylum	Class	VCT (n=3)	SMWCT(n=3)	AWCT(n=3)
Acidobacteria		<b>1.97</b>	<b>0.07</b>	<b>0.54</b>
	Acidobacteriia	1.87	0.06	0.42
	Solibacteres	0	0	0.06
	Holophagae	0.1	0.01	0.06
Actinobacteria		<b>1.19</b>	<b>1.44</b>	<b>1.42</b>
	Actinobacteria	1.15	1.44	1.41
	Thermoleophilia	0.04	0	0.01
Bacteroidetes		<b>45.2</b>	<b>32.63</b>	<b>27.01</b>
	Bacteroidia	0.97	25.11	9.75
	Cytophagia	2.54	3.1	2.03
	Flavobacteriia	<b>34.34</b>	1.36	1.81
	Sphingobacteriia	7.21	2.8	13.26
Chlamydiae		<b>0.01</b>	<b>0.12</b>	<b>0.23</b>
	Chlamydiia	0.01	0.12	0.23
Chloroflexi		<b>0.38</b>	<b>0.24</b>	<b>0.52</b>
	Anaerolineae	0.08	0.04	0.38
	Caldilineae	0.15	0.01	0.03
	Dehalococcoidia	0	0.06	0.04
Cyanobacteria		<b>0.48</b>	<b>0.03</b>	<b>1.31</b>
	Cyanobacteria	0.48	0.03	1.3
Deinococcus_thermus		<b>0.92</b>	<b>0.04</b>	<b>0.03</b>
	Deinococci	0.92	0.04	0.03
Elusimicrobia		<b>0</b>	<b>0</b>	<b>0.06</b>
	Elusimicrobia	0	0	0.05
Firmicutes		<b>8.52</b>	<b>29</b>	<b>7.16</b>
	Bacilli	1.09	5.95	1.26
	Clostridia	7.37	19.03	4.38
Fusobacteria		<b>0.38</b>	<b>0.63</b>	<b>0.49</b>
	Fusobacteriia	0.38	0.63	0.49
Lentisphaerae		<b>0.1</b>	<b>7.82</b>	<b>0.27</b>
	Lentisphaeria	0.01	1.69	0.21
	Oligosphaeria	0.09	6.13	0.06
Nitrospirae		<b>0</b>	<b>0</b>	<b>0.03</b>
	Nitrospira	0	0	0.03
Planctomycetes		<b>4.72</b>	<b>0.91</b>	<b>1.28</b>
	Planctomycetia	1.12	0.38	0.7

Proteobacteria	Phycisphaerae	3.6	0.53	0.57
		<b>23.29</b>	<b>16.92</b>	<b>47.11</b>
	Alphaproteobacteria	4.51	4.24	6.36
	Betaproteobacteria	9.23	3.15	29.28
	Deltaproteobacteria	1.15	3.14	5.27
	Epsilonproteobacteria	3.25	0.18	0.1
	Gammaproteobacteria	5.16	6.22	6.1
Verrucomicrobia		<b>10.22</b>	<b>1.65</b>	<b>5.7</b>
	Verrucomicrobiae	7.76	0.71	4.54
	Spartobacteria	0.9	0.03	0.02
	Opitutae	1.56	0.91	1.14

**Table 5.** The mean of the relative abundances expressed as percentage of the total number of sequences of the major fungal taxa in vermicompost tea (VCT), solid municipal compost tea (SMWCT), and agricultural waste compost tea (AWCT). Cells shaded red indicate the highest values for relative abundance

Phylum	Class	VCT (n=3)	SMWCT (n=3)	AWCT (n=3)
Ascomycota		<b>77.70</b>	<b>91.36</b>	<b>91.67</b>
	Arthoniomycetes	0.66	0.16	0.00
	Dothideomycetes	1.30	23.65	1.36
	Eurotiomycetes	13.98	24.96	22.27
	Lecanoromycetes	0.01	0.00	0.17
	Leotiomycetes	3.85	0.46	13.05
	Orbiliomycetes	0.00	0.00	0.10
	Pezizomycetes	8.54	1.88	6.52
	Saccharomycetes	0.03	0.81	0.11
	Schizosaccharomycetes	0.01	0.01	0.08
	Sordariomycetes	49.20	38.96	17.36
	Taphrinomycetes	0.00	0.00	0.01
	Unidentified	0.12	0.47	30.65
Basidiomycota		<b>15.38</b>	<b>4.29</b>	<b>1.05</b>
	Agaricomycetes	0.03	2.49	0.33
	Cystobasidiomycetes	0.00	0.00	0.04
	Dacrymycetes	0.00	0.00	0.00
	Malasseziomycetes	0.02	1.60	0.02
	Microbotryomycetes	0.02	0.02	0.00
	Pucciniomycetes	0.01	0.00	0.01
	Tremellomycetes	15.06	0.17	0.04
	Wallemiomycetes	0.08	0.00	0.05
Blastocladiomycota		<b>0.00</b>	<b>0.00</b>	<b>1.52</b>
	Blastocladiomycetes	0.00	0.00	1.52
Chytridiomycota		<b>2.06</b>	<b>0.03</b>	<b>1.99</b>
	Chytridiomycetes	2.06	0.03	1.99
	Monoblepharidomycetes	0.77	0.00	1.09
Entomophthoromycota		<b>0.01</b>	<b>0.01</b>	<b>0.75</b>



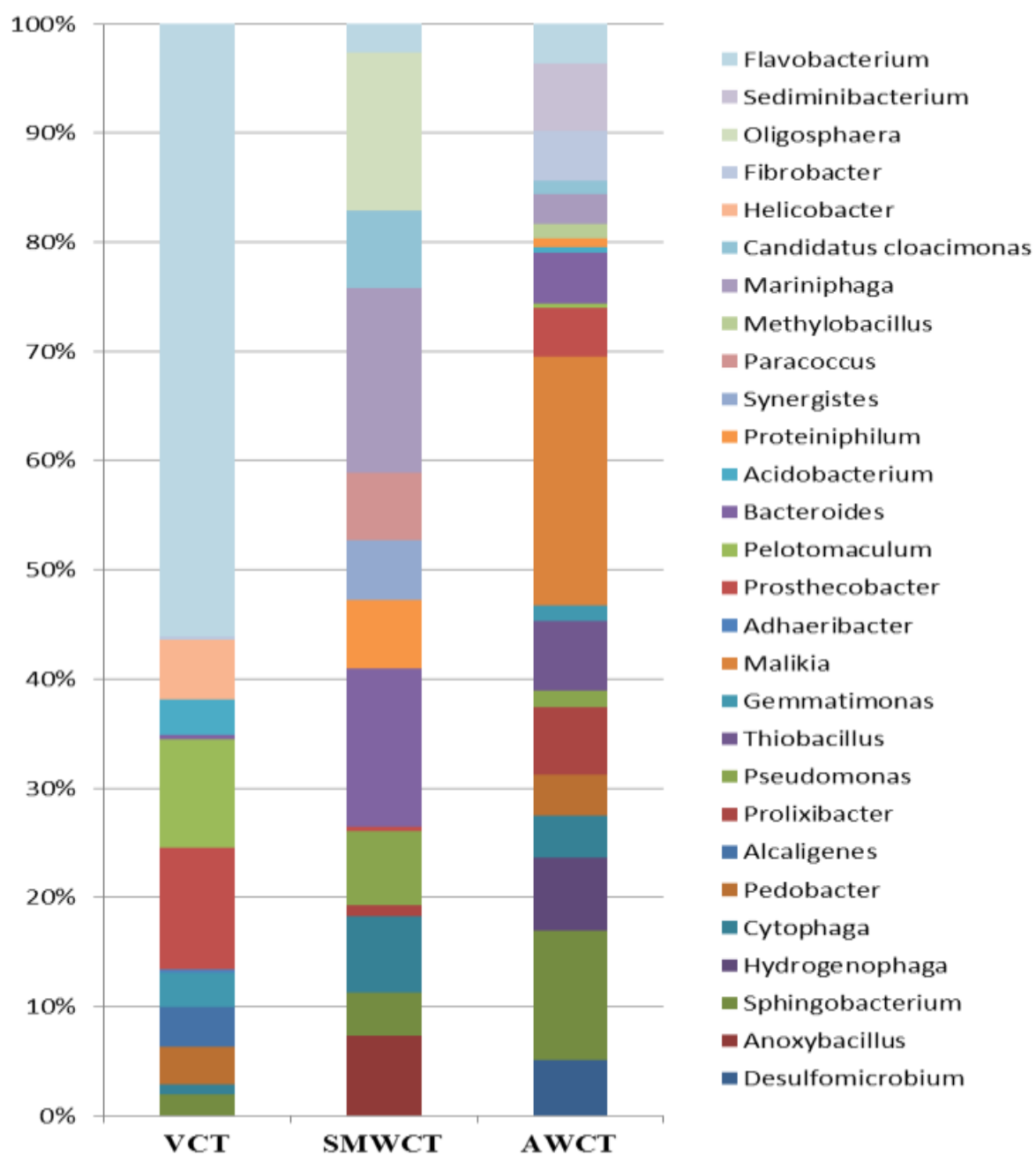
Glomeromycota	Basidiobolomycetes	0.01	0.01	0.75
		<b>0.04</b>	<b>3.87</b>	<b>1.35</b>
	Glomeromycetes	0.04	3.87	1.35
Neocallimastigomycota		<b>0.01</b>	<b>0.01</b>	<b>0.03</b>
	Neocallimastigomycetes	0.01	0.01	0.03

**Table 6.** Bacterial and fungal diversity indices for the three samples of each type of the non-aerated compost teas.

NCT <sup>a</sup> types	Bacterial 16S richness (S)	Bacterial 16S Diversity (H')	Fungal ITS richness(S)	Fungal ITS diversity (H')
VCT 1	677	2.7	177	2.96
VCT 2	547	2.4	60	2.52
VCT 3	561	2.5	59	2.57
SMWCT1	495	3.2	171	3.01
SMWCT2	525	3.9	56	1.36
SMWCT3	530	3.1	144	2.99
AWCT1	572	3.8	212	3.04
AWCT2	553	3.6	127	2.28
AWCT3	572	3.6	121	2.22

<sup>a</sup> vermicompost tea (VCT), solid municipal compost tea (SMWCT), and agricultural waste compost tea (AWCT). DNA was extracted from the NCTs 4 days after the last batch of each NCT was prepared.

The percentage contribution of the most common (top 10%) genera is shown in Figure 3. Genera including *Thiobacillus*, *Malikia*, *Hydrogenophaga*, *Desulfomicrobium* and *Prolixibacter* were only observed in AWCT whereas genera including *Oligosphaera*, *Paracoccus*, *Synergistes*, and *Anoxybacillus* were only observed in SMWCT. VCT appeared to be dominated by the genus *Flavobacterium* constituting about 32% of the top 10% of taxa. In contrast, the most even distribution of bacterial genera was observed in AWCT, an observation that is consistent with the alpha diversity statistics (Fig. 3).



**Fig. 3** Distribution of the dominant genera (top 10 %) based on relative abundance of percentage of sequences in VCT (vermicompost tea), SMWCT (solid municipal waste compost tea) and AWCT (agricultural waste compost tea).

#### 4.4. Discussion

Application of each type of NCTs to the soil of potted potato plants reduced the severity of bacterial wilt disease compared to the non-treated plants (Fig.1, Table 3). These compost teas varied in their physico-chemical and biological characteristics as influenced by the composition of the compost used to make each tea. The application of high-throughput sequencing in this study revealed that the bacterial and fungal communities across the three NCTs varied markedly. While the microbial data represents the community at one time point, this provides an example to compare the NCTs used. The variable level of bacterial wilt suppression observed in this experiment was also associated with the interval between the date of NCT application and the date of inoculation with the target pathogen.

AWCT suppressed disease symptom development to a greater degree than SMWCT or VCT for all initial application times evaluated. The choice of composting substrates is known to have a significant impact in producing disease suppressive compost teas for certain pathosystems (Scheuerell and Mahaffee, 2006; Al-Dahmani *et al.*, 2003). Generally, composts (and presumably their watery extracts) must have attributes within a consistent range to be used successfully in biological control of pathogens of horticultural crops (Hoitink *et al.*, 1997). Biotic and physico-chemical properties of the parent compost and their NCTs also play a major role in disease suppression. For example, the C:N ratio of parent composts should not be too high to prevent nitrogen immobilization, which results in competition between the microbes and plants for nutrients in the soil, or too low to prevent the release of phytotoxic compounds such as ammonia to the soil (Gao *et al.*, 2010). In this trial, the AWCT made from both animal and plant-based substrates in equal proportion, had

a relatively low EC, neutral pH, and C: N ratio (19:1) of the parent compost, which appeared to be in the optimum C:N range (<25:1) (Kuo *et al.*, 2004). Presumably these characteristics favour microbial activity in the soil (Moral *et al.*, 2009) that in turn suppresses the severity of soil borne disease (Hoitink *et al.*, 1997). In contrast, SMWCT and VCT had a higher concentration of extractable cations and EC, and very low C: N ratio in the respective parent compost sources. The higher wilt severity which occurred on plants treated by these two NCTs might be due to less favourable conditions for growth and proliferation of antagonistic microorganisms, due to high salt content and low C:N ratio as previously reported for different pathosystems (Aryantha *et al.*, 2000). Hoitink *et al.* (1993) showed that application of SMWC with a high salt concentration at planting time reduced soybean yield due to promotion of root rot caused by *Phytophthora* spp. Similarly, Md Islam and Toyota (2004) reported that tomato plants treated with bark and coffee compost with high Ca and Mg concentration, had a higher incidence of bacterial wilt than plants treated with farm yard manure (FYM) containing significantly lower concentration of extractable cations. Therefore, it is evident that the quality of parent composts influences the degree to which NCTs suppress bacterial wilt.

It is known that the biotic components of composts, originating from the organic waste composts, contribute to the suppression of wilt disease on potato and other hosts caused by the soil-borne *R. solanacearum* (Ding *et al.*, 2013). Few studies, however, have investigated the diversity of microbial communities in compost teas in relation to their potential to suppress potato wilt and other diseases. The greater bacterial and fungal diversities of AWCT relative to the other NCTs might have contributed to the higher level of disease suppression observed. Md Islam and Toyota (2004) incorporated composted farm-

yard manure into soil and observed enhanced microbial activities and a greater diversity of bacterial and fungal communities. This soil amendment resulted in poor survival in terms of the population of *R. solanacearum*. A diverse microbial community in the soil contains a diverse array of functional properties which places pressure on the population of soil-borne plant pathogens (van Elsas *et al.*, 2012). One example is the work of Shiomi *et al.* (1999) who found that soil with a high microbial diversity was suppressive to *R. solanacearum*.

In recent years, genera not explored previously as biological control agents (BCAs) for bacterial wilt have been identified from diverse sources (Yuliar and Toyota, 2015). Our analysis revealed genera that were identified in one type of NCT but not another and also dissimilarities in microbial composition that may have affected the biological activity and function of the communities. Even though common genera used as BCAs - *Bacillus*, *Pseudomonas*, and *Burkholderia* - were identified in all NCTs and are known to be antagonistic to *R. solanacearum*, their frequency was variable and they were present within a background microbial community that varied across NCTs effecting different levels of disease suppression. Moreover, the species and strains of the genera present were not studied, although these are known to influence the efficacy of biological control. It is apposite to note that biological based control measures of crop disease are mostly influenced by strain specificity, among other factors. For instance, Thomas and Upreti (2014) studied three isolates of the endophytic bacterium *Bacillus pumilus* isolated from grape and watermelon. Only one isolate from grape inhibited the *in vitro* growth of *R. solanacearum* while the two isolates from watermelon were not consistently antagonistic. Apart from chemical and biological attributes of NCTs, we suggest that strain specificity might have also played a role in the apparent variability in disease suppression of NCTs tested in the different times of application.

This study showed that the greatest suppression of bacterial wilt was achieved when the initial application of NCT was timed to coincide with tuber planting and inoculation. In a related study, Anith *et al.* (2004) reported that application of treatments including plant growth promoting rhizobacteria (PGPR) strains and mixtures of organic amendments at the time of seeding and a week before inoculation with *R. solanacearum* significantly reduced bacterial wilt in tomato. Plants first treated with NCTs 1 week after inoculation (curative) showed significantly greater disease than application on or before inoculation. *R. solanacearum* survives in the soil and replicates prior to entering the tuber buds/eyes or lateral roots via natural openings or wounded tissues (Álvarez *et al.*, 2008). Although the fate of *R. solanacearum* after treatment application was not rigorously examined in this research, we hypothesise that the mechanism of disease suppression is competitive exclusion of the pathogen from the infection court and/or direct antagonism of the pathogen population by the microbes present in NCT. In some cases biological control agents work through the production of specific metabolites and it is possible that similar mechanisms were also present in our system. However, both this, and other studies (Scheuerell and Mahaffee, 2006; Palmer *et al.*, 2010) indicate that the presence of a diverse microbial community is essential for effective suppression of disease. It is also acknowledged that different results might have been observed if the experiment had been conducted with non-autoclaved field soil because its associated microbial community may have interacted with that present in the NCTs.

We conclude that compost tea produced as a non-aerated, water ferment of composted agricultural wastes comprising maize and wheat straw, chopped grasses, ash, and animal manure substrates has potential to be used as part of an integrated disease management

strategy. The microbial diversity of NCT, especially bacterial diversity, appears to contribute to the level of disease suppression observed. Further studies are needed to enhance the efficacy of compost teas more generally by elucidating the mechanism/s of action.



# Chapter 5

## TREE-DERIVED GUM ENHANCES THE EFFECT OF NON-AERATED COMPOST TEA IN SUPPRESSING BACTERIAL WILT OF POTATO

### 5.1. Abstract

Three non-aerated compost teas (NCTs): AWCT (agricultural waste compost tea), SMWT (solid municipal waste) and VCT (vermicompost tea) were combined with gum myrrh and oppoponax that were separated from their respective oleo-gum-resin exudates. Each treatment was applied directly to potato tubers and as a soil drench prior to planting tubers in pots, followed by three applications to above-ground plant tissues. Stems of 15-20 cm tall plants were inoculated with *Ralstonia solanacearum* after the first foliar spray of an NCT. Bacterial wilt reached maximum severity by 60 days after inoculation (DAI) in the water-treated control treatment and all NCT treatments resulted in a lower mean disease severity index (DSI) than the control treatment. AWCT resulted in a significantly higher suppression of bacterial wilt compared to NCT made from both vermicompost and solid municipal compost tea. AWCT combined with gum myrrh resulted in a lower DSI (46% by 60 DAI) and area under the disease progress curve (AUDPC) than all other treatments, except for the AUDPC for VCT combined with gum myrrh. The results demonstrated that the addition of plant-derived gum to compost tea as an adjuvant can enhance the suppression of bacterial wilt in potato.

## 5.2. Introduction

Bacterial wilt, caused by the soil-borne pathogen *Ralstonia solanacearum*, reduces the yield of many solanaceous crops in tropical and subtropical regions (Champoiseau *et al.*, 2009; Hayward, 2000). Potato (*Solanum tuberosum* L.) is mainly affected by the widespread race 3, biovar 2 (R3bv2) sub group that causes major economic losses globally (Álvarez *et al.*, 2010a; Messiha *et al.*, 2007). Management of bacterial wilt using existing disease-control practices has been difficult. As the pathogen has a wide range of volunteer hosts (Tusiime *et al.*, 1998) and can persist in the soil for a long time (van Elsas *et al.*, 2000), cultural management practices (mainly crop rotation) have not always been effective (Lemaga *et al.*, 2001). Moreover, options to control bacterial wilt of potato with chemical pesticides, such as application of the soil fumigant methyl bromide, are either no longer available or their use has been restricted (Messiha *et al.*, 2007). Therefore, development of effective, safe and practical management options for potato bacterial wilt are required.

As part of the broader concept of microbial-mediated disease management, compost teas have been shown to be beneficial in controlling plant disease (Deepthi and Reddy, 2013; Siddiqui *et al.*, 2008). Compost teas produced from different types of compost inhibit plant pathogens to varying degrees (Diénez *et al.*, 2006; Pane *et al.*, 2012) and their efficacy in certain pathosystems has been too variable to be used reliably for disease management (Scheuerell and Mahaffee, 2006).

Attempts have been made to increase effectiveness of compost teas used for disease suppression by adding nutrients during production to enhance microbial populations (El-Haddad *et al.*, 2014; Scheuerell and Mahaffee, 2004), or post production addition of adjuvants (Scheuerell and Mahaffee, 2006; Ketterer *et al.*, 1992). Scheuerell and Mahaffee

(2006) found the application of compost tea, with gum karaya as an adjuvant, significantly reduced gray mold disease on geranium compared to tea with no adjuvant.

Biopolymers have been reported to suppress plant disease either applied alone (Luiz *et al.*, 2012) or in combination with biocontrol agents to improve the degree of disease suppression (Lima *et al.*, 2005). Multiple mechanisms of actions of biopolymers are known in biological-based plant disease management, including their use as carriers in formulations (Chen *et al.*, 2013a) and as adjuvants whereby they slow the rate of microbial desiccation and stabilize the activity of microorganisms (Hynes and Boyetchko, 2006; Lima *et al.*, 2005). Most biopolymers such as arabic gum, guar gum, karaya gum, locust bean gum, and azadirachta gum are processed from plants (Lima *et al.*, 2005; Chen *et al.*, 2013a; Bill *et al.*, 2014). Their availability for use in crop protection could be limited given the high demand and use of these products in the pharmaceutical and food industries (Sorokulova *et al.*, 2015). Therefore, there is a need to evaluate other sources of adjuvants for their potential as additives in crop disease management. Oleo-gum resinous exudates, mainly myrrh and opoponax, collected from trees of *Commiphora* species of the *Burseraceae* family, are predominantly found in eastern Africa (Lemenih and Kassa, 2011; Tadesse *et al.*, 2007). They contain water-soluble gum, volatile oils and alcohol-soluble resins (Hanuš *et al.*, 2005). The water-soluble fraction of exudates from *Commiphora* species are sources of crude polysaccharides with a range of applications, including as thickening and coating agents (Lemenith and Teketay, 2003).

In our previous study (see Chapter 4), compost teas were applied weekly as both a soil drench and to above ground plant parts of potato (Mengesha *et al.*, 2017). There was a 2.5-fold reduction in the severity of bacterial wilt (from 83% in non-treated controls to 33% in

the best compost tea treatment); however, greater disease suppression is desired in order to increase the likely effect in a field situation with variable environmental conditions. Therefore, additional trials are presented here to examine other factors to enhance compost tea efficacy, primarily application of NCT directly to tubers and the addition of gum additives to NCTs. In this study, we hypothesised that tubers treated with compost tea and carbohydrate gum additive would result in an enhanced suppression of bacterial wilt symptoms in potato plants relative to water-treated tubers. Therefore, the aim of this study was to evaluate three types of NCTs, applied alone or in combination with a tree gum, for suppressing the development of bacterial wilt in potato shoots emerging from tubers planted in pots in a greenhouse experiment.

### 5.3. Materials and Methods

#### *Bacterial isolate and inoculum preparation*

The race (r) 3 biovar (bv) 2 (virulent) strain of *R. solanacearum*, isolated from tomato as described previously by Mengesha *et al.* (2017), was cultured on 2, 3, 5-triphenyltetrazolium chloride agar (TZC) medium (Kelman, 1954) for 48 h at 28°C. Colonies with morphological characters of mucoid, reddish and irregularly shaped colonies, and having a central white colour typical of virulent *R. solanacearum* were identified. An individual colony was transferred to nutrient broth media for multiplication. The inoculated media was incubated for a further 48 h at 150 rpm on orbital shaker at room temperature prior to use in the bioassay.

#### *Preparation and characterization of compost tea*

Mature samples of agricultural waste compost (AWC), vermicompost (VC), and solid municipal waste (SMWC) were used for preparation of non-aerated compost teas (NCTs) according to the procedure outlined by Koné *et al.* (2010). Briefly, plastic buckets (30 L capacity) were used to prepare the different batches of NCTs in 1:5 ratios (w/v) using agricultural well water for a period of 14 days. Layered muslin cloth was used to filter the compost teas. The compost teas were stored in a cold room (5-8°C) until used in the bioassay. Physico-chemical and microbial characterisation of both the parent compost sources and the NCTs has been previously described and are summarized in Table 1 (Mengesha *et al.*, 2017) The C: N ratio and EC measurements for AWC were found to be in the acceptable range for good quality composts, whereas the ratio for SMWC and VC was sub-optimal. The three NCTs generally had higher bacterial diversity (H) and richness (S) than fungal OTU's. AWCT had a higher bacterial diversity H and fungal richness S than the other two NCTs. Similarly, VCT showed higher bacterial richness but the fungal richness did not significantly vary between the NCTs as reported in Chapter 4.

**Table 1.** The pH, electrical conductivity (EC) and C: N ratio of the composts used to make the non-aerated compost teas (NCTs) and the pH, EC, and fungal and bacterial diversity indices of the corresponding NCTs

Compost sources <sup>a</sup>	pH (1:2.5 H <sub>2</sub> O)	EC (mS/cm)	C:N	16S H	16S S	ITS H	ITS S
AWC	7.1	1.08	19.8				
SMWC	8.5	19.8	3.1				
VC	7.4	11.6	3.7				
<i>Non-aerated compost tea</i>							
AWT	7.5	0.64		3.7	567	2.5	153
SMWT	7.8	10.3		3.4	517	2.4	123
VCT	7.8	11.6		2.5	595	2.6	98

<sup>a</sup> compost sources and corresponding nonaerated compost tea: AWC—agricultural waste, SMWC —solid municipal waste compost, and VC— vermicompost. 16S and ITS (H)—

bacterial and fungal diversities, respectively. 16S and ITS (S)—bacterial and fungal richness (counts of the OTUs), respectively. This information has been adapted from (Mengesha *et al.*, 2017) and presented here for reference.

#### *Extraction of gum from tree exudates*

Staff from the School of Pharmaceutical Chemistry, Addis Ababa University collected exudates by tapping trees identified as *Commiphora myrrha* (Nees) and *Commiphora erythraea* var. *glabrescens*, which are commonly known as myrrh and opoponax, respectively. The trees sampled were from the south- eastern dry land area of Ethiopia (Daniel Bisrat, *personal communications*). The exudates were in the form of oleo-gum-resins that were slightly sticky and dark brown in appearance. The exudates were partially dry at the time of separation of the components.

A three-step extraction process was followed to separate the water soluble gum component from the exudates, based on a method modified from that described by Gebrehiwot *et al.* (2015). Briefly, 200 g of exudate was mixed with 300 ml water to extract the essential oils by hydro-distillation in a clavenger apparatus for 5 h. After the oil was removed, the remaining gum resins were extracted by successive maceration in 96% ethanol (four times), each for 30 min, and resinoids were obtained as a crude extract by removing the solvent under vacuum in a rotary evaporator. Finally, the remaining marc was further re-extracted with distilled water three times, each extraction for 2 h, separately filtered with filter paper, filtrates pooled, and dried at 105°C in an oven, to yield a water-soluble gum fraction that was then added to the test compost teas. The whole procedure was conducted three times

to collect the desired amount of product for the bioassays, where they are designated opoponax gum 1 (G1) and *myrrh* gum 2 (G2).

### *Greenhouse experiment*

Plant material, inoculation and treatment setup:

Potato (*Solanum tuberosum* L.) of variety *Jalene* was used in the trial. Single sprouted tubers were planted in 20 cm diameter pots which were filled with a mixture of field soil, agricultural waste compost, and sand (2:1:1, respectively). The planting medium was autoclaved at 121°C for 2 h and cooled before tubers were planted.

The three types of NCT evaluated were prepared from the following compost types: agricultural waste (AW), solid municipal waste (SMW), and vermicompost (VC). Each treatment (Table 2) comprised multiple applications of each NCT, NCT amended with gum G1 or G2, or water. The sequence of applications was (1) dressing tubers by soaking in the respective treatment for 3 h ahead of planting, with treated tubers left to dry for 2 h (2) drenching the soil in the pot just before planting; and (3) application of a spray to above the ground plant part to run-off at three time points. The treated tubers were left to dry for 2 h and the foliar sprays were applied 2 days before inoculation with *R. solanacearum* when the plants had reached 12–15 cm height, and then twice, 2 and 3 weeks after inoculation. A sample of *R. solanacearum* culture in nutrient broth media was adjusted to a concentration of 10<sup>9</sup> cfu/ml and a micro-syringe containing 4 ml of bacterial suspension was injected in to the stems of each plants just beneath the first node from the soil according the method of

Winstead and Kelman (1952). The experiment was maintained in the temperature range of 25–32°C and the relative humidity was approximately 70%. The experimental design was randomised complete-block design of 11 treatments with three replications (blocks).

**Table 2.** List of compost tea treatments used to evaluate the suppression of potato bacterial wilt in the greenhouse experiment. Each pot received 200 ml compost tea for the soil drench, and the gum concentration in the compost tea was 0.05% (w/v) for all applications

Treatment	Description
AWT	Agricultural waste compost tea
SMWT	Solid Municipal waste compost tea
VCT	Vermicompost tea
AWT-G1	Agricultural Waste Compost tea & Gum-1 mix
SMWT-G1	Solid Municipal waste compost tea & Gum-1 mix
VCT-G1	Vermicompost tea & Gum-1 mix
AWT-G2	Agricultural Waste Compost tea & Gum-2 mix
SMWT-G2	Solid Municipal waste compost tea & Gum-2 mix
VCT-G2	Vermicompost tea & Gum-2 mix
Control	Inoculated control

Disease assessment:

Final bacterial wilt severity was evaluated at 60 days after inoculation (DAI) with *R. solanacearum*. Yellowing and stunting of the aboveground plant parts, typical of the symptoms of bacterial wilt were first observed at 20 DAI. Disease severity for each potted plants per treatment was assessed 26, 33, 40, 47 and 55 days after inoculation using a 0–4 scale (Kempe and Sequeira, 1983), where 0=no symptoms, 1= 1–25% of the foliage wilted, 2=25–50% of the foliage wilted, 3=50–75% of the foliage wilted and 4=75–100% of the foliage wilted.

Data analyses:



The mean disease rating was calculated from the severity scores for each of the three replicates per treatment. This mean was then expressed as a percentage of the maximum possible score (4) to express disease severity on a scale of 0–100 (Winstead and Kelman, 1952). Disease severity index (DSI) (%) assessed at the different times after planting was used to calculate the area under the disease progress curve (AUDPC) by the method of Campbell and Madden (1990). The presence or absence of treatment effects was tested using one way analysis of variance with the GLM procedure for the final disease severity index and AUDPC response variables using the SAS software program (SAS Institute, 2011). When effects of treatments were significant ( $P < 0.05$ ), means were compared using Fisher's protected least significant difference (LSD) test.

## 5.5. Results

### *Characters of the gum sources (exudates)*

The yields of the three components showed that opoponax had a slightly higher percentage of both the essential oil and the resinous component, whereas myrrh had higher slightly higher percentage of the water-soluble fraction (Table 3).

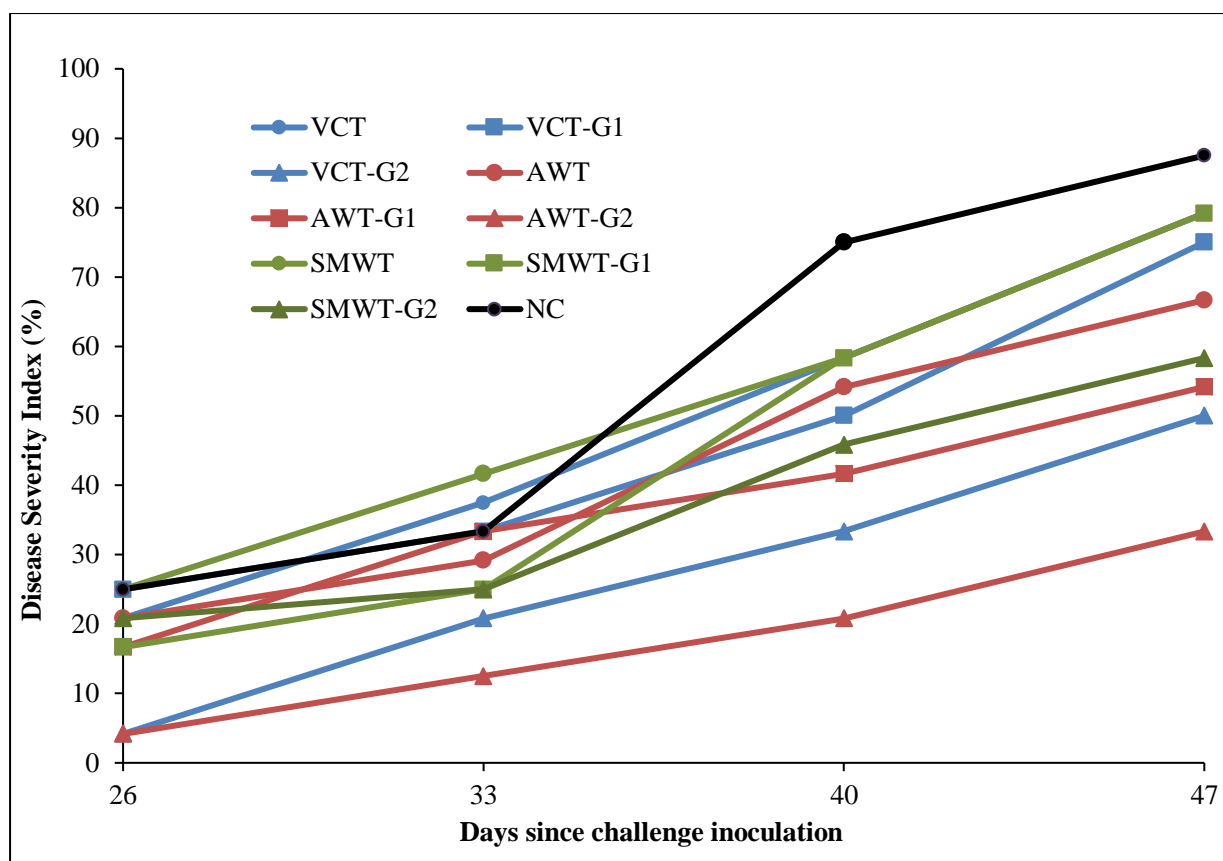
**Table 3.** Essential oils, resins and gum yields of *Gum Opoponax* and *Gum Myrrh*

Source	Essential yield w/w)	oil (%, yield (%, w/w)	Resin yield (%, w/w)	Gum yield (%, w/w)
Opoponax (G1), <i>Commiphora myrrha</i> (Nees)	4.2		22.9	58.6
Myrrh (G2), <i>Commiphora erythraea</i> var. <i>glabrescens</i>	3.8		21.1	62.0

### *Effect of treatments on bacterial wilt development*

Symptoms of bacterial wilt were well established by 60 DAI and all control plants treated with water were dead (DSI=100%). In general, the three NCTs without added gum (VCT, AWCT, SMWT) showed higher DSI at all assessment times (Fig 1) compared to the same treatment with either gum.

At 26 DAI, a mean DSI of 4.2 % was observed in both the AWT-G2 & VCT-G2 treatments which was significantly lower ( $p<0.05$ ) than for all other treatments in which mean DSI was 17– 25% (Fig. 1). At 33 DAI, the mean DSI was 42 and 33% in the SMWT and water-treated pots, respectively whereas the mean DSI in the AWT-G2 & VCT-G2 treatments was 13 and 21%, respectively, significantly lower than the rest of treatments ( $p<0.05$ ). By 40 DAI, the mean DSI in the water-treated control was 75%, which was significantly higher ( $p<0.05$ ) than the means for all other treatments. Mean DSI for the AWT-G2 treatment at this time was 21%. On the second last assessment date (47 DAI), the mean DSI in the water-treated control reached 88%. AWT-G2 and VCT-G2 had a mean DSI of 33% and 50%, respectively, which was significantly lower than all other NCT treatments ( $P<0.05$ ), although the mean for VCT-G2 was statistically similar to that for AWT-G1 (54%) and SMW-G2 (58%) (Fig. 1).



**Fig 1.** Mean bacterial wilt severity (%) at different times after inoculation of potato variety *Jalene* with 4 ml of  $10^9$  cfu/ml pathogenic *Ralstonia solanacearum* injected into the stem of each plant. Disease severity for each potted plant (3 plants per pot) was assessed using a 0-4 scale and the mean value expressed as a percentage of the maximum possible score (4). VCT = vermicompost tea, AWT= agricultural waste tea, SMWT =solid municipal waste tea, G1 indicates opoPONAX gum, G2 indicates myrrh gum and no label indicates compost tea without gum. NC is water control.

The final disease assessment at 60 DAI revealed that the mean DSI of control plants (NC) was significantly greater than the mean DSI of all other treatments (Table 4). AWTG2, VCTG2, and AWTG1 resulted in a lower AUDPC than the control and the most of treatments (Table 4)

All plants treated with NCT without gum (VCT, SMWT and AWT) and two of those with opoponax gum added (VCT-G1 and SMWT-G1) had mean DSIs in the range 79-88% (Table 4). Addition of myrrh gum (G2) to both SWMT and VCT reduced DSI to 58-67%, and resulted in mean DSIs that were significantly lower than the means for the same teas without gum or with opoponax gum (G1) (Table 4). AWC amended with opoponax gum (G1) also resulted in a similar mean DSI to these treatments, whereas AWC amended with myrrh gum (G2) resulted in a mean DSI lower than any other treatment and resulted in a 54% reduction in mean DSI relative to the water-treated control (Table 4).

**Table 4** The effect of non-aerated compost with or without gum additives on bacterial wilt severity of potato 60 days after inoculation in a greenhouse experiment. Refer to Table 2 for treatment codes. Means sharing the same letter within columns are not significantly different at  $P = 0.05$ .

Treatment	DSI <sup>a</sup>	AUDPC <sup>b</sup>
VCT-G1	87.5b	1390 abc
SMWT	87.5b	1508 ab
VCT	87.5b	1492 ab
SMWT-G1	83.3b	1376 abc
AWT	79.2b	1288 abc
SMWT-G2	66.7c	1098 bcd
VCT-G2	58.3c	864 ed
AWT-G1	58.3c	1055 dc
AWT-G2	45.8d	586e
NC	100 a	1668a

<sup>a</sup> DSI = Mean Disease Severity Index =  $S_{nr} \times 100 / N_{pr} \times M_{sc}$ ; where  $S_{nr}$  is the sum of numerical ratings of disease severity (0 to 4 scale),  $N_{pr}$  is the number of plants rated and  $M_{sc}$  is the maximum possible numerical rating (4).

<sup>b</sup> AUDPC is the area under the disease progress curve for each treatment.

## 5.6. Discussion

This study confirmed our previous findings that application of NCT made from agricultural waste compost resulted in significantly higher suppression of bacterial wilt in potato compared to NCT made from both vermicompost and solid municipal waste compost tea. Moreover, the addition of myrrh gum as an adjuvant enhanced the efficacy of NCTs in this experiment.

Here we observed an earlier appearance of disease symptoms and higher disease severity on water-treated plants and in most of the treatments when compared to our previous findings using same experimental conditions (Mengesha *et al.*, 2017). Kempe and Sequeira (1983) found that disease progress was more rapid in potato plants emerged from biocontrol-treated tubers followed by stem inoculation by pathogenic *R. solanacearum* than plants exposed to the pathogen by soil inoculation. Introducing the pathogen directly through stem inoculation was likely to result in a high disease severity because the pathogen propagules are delivered rapidly and directly to the vascular tissue. In pioneer study to evaluate the pathogenicity of *R. solanacearum*, Kelman (1953) reported that stem inoculation of the pathogen resulted in a rapid development of bacterial wilt in tomato.

In this trial, agricultural waste compost tea combined with gum myrrh suppressed development of bacterial wilt symptoms throughout the growing period. The AW parent compost, relative to VC and SMWC, had optimum quality levels of EC, pH, and C: N.

Moreover, AWCT had a higher bacterial Shannon diversity index than the other two NCTs. This index also indicates evenness in the abundance of taxa. It is postulated that these physico-chemical and biological features of AWCT promoted higher anti-microbial activity and thus greater disease suppression relative to the other types of NCTs evaluated. It has been shown that incorporation of diverse microbial communities to the growing medium such as soil shifts the microbial community structure in relation to the population of the pathogen (Hadar, 2011; Liu *et al.*, 2015) or induces resistance (Hadar and Papadopoulou, 2012).

When the site of compost tea treatment and plant tissue inoculated are physically separated, then disease suppression is most likely mediated by induced resistance, as suggested by Yogeve *et al.* (2010) who found that compost-grown cucumber and melon plants that were inoculated with *Botrytis cinerea* showed significantly smaller necrotic areas than those of peat-grown plants. Our findings are consistent with this and previous reports of lower wilt incidence relative to control treatments when potato tubers were treated by dipping them in a suspension of a biocontrol agent, chemical compounds or plant extracts before planting and resulted followed by inoculation of emerged plants with *R. solanacearum* (McLaughlin *et al.*, 1990; Hassan *et al.*, 2009; Gado, 2013).

The effect of gum myrrh has enhanced the efficacy of all three NCTs compared to NCTs without amendment. Myrrh had a 3.4 % higher proportion of the gum fraction than oppoponax; however, their essential oils and resinous fractions were similar. Sudisha *et al.* (2009) reported that a field trial of pearl millet seed treated with *Acacia arabica* gum alone resulted in 48% of disease protection against downy mildew. The value further increased to

96% when the gum was combined with synthetic fungicide metalaxyl 35 SD, which provided 96.4 % when applied alone.

In principle, natural polymer additives are likely to influence the survival rate and stability of the microorganisms (John *et al.*, 2011; Ma *et al.*, 2015). The gum component in oleo-gum-resin is chemically composed of mixtures of polysaccharides and hence could be used as an additive as opposed to the volatile and resinous fractions that are documented for their strong antimicrobial properties against range of aerobic and anaerobic bacteria (Raja *et al.*, 2011; Weckesser *et al.*, 2007).

The physico-chemical and microbial diversity qualities of agricultural waste derived compost tea combined with the high percentage of carbohydrate containing myrrh was most likely to be a suitable combination for enhanced disease suppression. Our finding warrants further studies on gum biopolymers as adjuvants including investigating the effect of variation in concentration of gum fraction with compost tea. Repeated *in situ* studies to evaluate combination of compost with gum aid optimisation of a compost tea solution for suppression of bacterial wilt.

# Chapter 6

## SUCCESSION OF MICROBIAL COMMUNITIES IN NON-AERATED COMPOST TEAS FROM AGRICULTURAL WASTES DURING UNDERGROUND COMPOSTING

### 6.1. Abstract

Non-aerated compost tea (NCT) is used by some organic farmers primarily to reduce disease in horticultural crops. There is a perception that these extracts also enhance soil and plant nutrient status because of the presence of beneficial microbes. Studies on compost tea previously highlighted the presence of diverse microbial populations. Here, we evaluated NCTs that were made from compost samples of three different ages (21, 42, 63 days after compost initiation) and were produced from two types of farm residues: agricultural waste (AWCT) and coffee husk dominated compost (CHCT) in an on-farm composting system. The physico-chemical features of the composts used to make teas were described. Next generation amplicon sequencing was used to characterise the microbial community composition and structure of the NCTs. The most abundant bacterial phyla, such as *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and *Chloroflexi*, were identified at all stages of composting but with marked shifts in their relative abundances. Fungal taxa, mainly *Ascomycota*, *Basidiomycota*, *Cryptomycota*, *Entomophthoromycota* and



*Glomeromycota*, were abundant phyla, also with variable relative abundances over time. Analysis of Similarity (ANOSIM) revealed that there were significant differences in community structure for both bacterial and fungal communities over time; however, pair wise comparisons suggested that teas from some composting stages were similar in microbial community structure. Bacterial diversity, but not richness, was higher in the 63-day sample of AWCT than the 21 and 42 day samples. In CHCT, there were no significant differences in either diversity or richness across the stages of composting. This study highlighted that similar phyla of bacteria and fungi are present in NCT made from different stages of composting. Despite some differences in the relative abundance of a small number of taxa, it appears that any of these stages of composting could be used for NCT production.

## 6.2. Introduction

Composting is a process of decomposition of organic materials in controlled conditions that enables the bio-conversion of industrial and agricultural wastes into valuable farm resources (Ryckeboer *et al.*, 2003b; Misra *et al.*, 2003). In the process, organic waste substrates are converted into dark brown to black colloidal masses and when added to the soil confer beneficial effects to the growth of plants (Litterick *et al.*, 2004). In addition, the process of composting has a significant positive environmental impact as it removes wastes that might otherwise occupy valuable landfill space (Neher *et al.*, 2013; Kuo *et al.*, 2004), which is a major problem both in developed and developing countries where accumulation of wastes is an environmental concern (Shemekite *et al.*, 2014; Misra *et al.*, 2003).

Apart from its role as a soil conditioner and source of organic fertilizer in improving soil chemical and biological properties, it is well documented that compost amendments can suppress soil borne plant disease in some situations (Alfano *et al.*, 2011; Termorshuizen *et al.*, 2006; Hoitink *et al.*, 1997). Similarly, composted organic wastes can be used to make preparations with water known as compost tea, which have been of interest to horticultural and ornamental crop growers as protectants against soil borne and foliar diseases (Siddiqui *et al.*, 2015; Evans and Percy, 2014). In many reports, the efficacy of compost teas was influenced by the type of the pathosystem (the host-disease interaction) and the quality of compost that in turn is influenced by the substrate type, physico-chemical and microbiological compositions (Koné *et al.*, 2010; Haggag and Saber, 2007). Therefore, studying the biological communities in the compost extracts known as “compost teas” is important to understand the effects of the consortia of microorganisms applied on plant and soil surfaces as a means to suppress plant pathogens and/or as bio-fertilizers.

Four phases of composting are known to occur, based on the temperature within the compost: mesophilic (10–42°C), thermophilic (45–70 °C), secondary mesophilic (10–42°C) and curing or maturation phases (ambient temperature) (Ryckeboer *et al.*, 2003b; Hoitink *et al.*, 1997). Classes of microorganisms that are involved in composting are influenced by factors such as chemical composition of the starting material and physical characteristics including temperature, aeration, moisture, C: N and pH (Chandna *et al.*, 2013; Ishii and Takii, 2003). Composting methods are broadly categorized as (1) small-scale, traditional or (2) large-scale and rapid practices (Misra *et al.*, 2003). The latter methods are controlled by aerobic processes that employ frequent mechanical turning with forced aeration and typically result in a high temperature period (Partanen *et al.*, 2010; Ryckeboer *et al.*, 2003a).

According to Misra *et al.* (2003), traditional methods are also known as on-farm composting methods and are conducted in anaerobic or passively aerobic systems through infrequent turning or the use of perforated holes. Such methods are characterised by long composting times, low temperature, and are processed either in heap or pit structures (St. Martin and Brathwaite, 2012; Litterick *et al.*, 2004).

Studies have indicated that, where composts are used as a soil amendment to control plant disease, mature and stable compost showed higher suppressive efficacies and lower phytotoxicity than products from the early stages of composting (Tang *et al.*, 2006; Hoitink *et al.*, 1997). Likewise, a review by Scheuerell and Mahaffee (2002) found that effective compost teas were prepared from cured and stable compost materials that are considered good quality compost. However, Palmer *et al.* (2010) found that aerated compost tea prepared from compost in the early secondary mesophilic stage in a controlled aerobic composting process had higher microbial diversity that was correlated with higher pathogen suppression than tea from mature compost samples. Therefore, both the type of substrate and stage of composting appear to be critical factors that influence the microbial communities and effectiveness of the final compost teas. A greater understanding of these factors is needed to interpret the resulting composition and role of microorganisms in the effectiveness of compost teas.

In Ethiopia, common composting substrates are agricultural wastes that are generated from field crop residues and animal manures from the mixed farming systems (Nigussie *et al.*, 2015). On-farm pit methods are prevalent in the central highland areas where composting is a common practice. Similarly, coffee husks derived from small to large scale wet and dry

coffee processing plants are environmental pollutants in coffee growing regions (Shemekite *et al.*, 2014; Kassa *et al.*, 2011). Composting is one solution to utilise this waste.

This study was designed to characterise the biotic component of non-aerated compost tea (NCTs) and to elucidate the shifts in microbial community composition and structure of NCTs prepared on-farm from compost at various stages of maturity and using readily available agricultural wastes. The changes in bacterial and fungal communities in the NCTs produced from successive stages of composting were evaluated using next-generation amplicon sequencing of extracted genomic DNA. Microbial taxa that were differentially abundant at different stages of composting are described.

### 6.3. Materials and Methods

#### *Composting system*

Composting was conducted at an experimental field station of the Ambo Plant Protection Research Centre, located at 8°57'N, 37°52'E, Ethiopia. Two separate composting systems were established with different substrate compositions: agricultural waste compost (AWC) and coffee husk mixed with animal manure, in underground pits with passively aerated conditions, a method similar to the Indian Indorepit system (FAO, 1980), with some modification (Buzayehu Tola, Personal Communication).

For the AWC, the substrates were composed of plant (maize and wheat straw, cowpea straw, chopped grasses), animal waste (cow dung), wood ash and forest soil. The fresh plant materials were crushed to reduce size, and then spread around the composting sites and left to wilt for 2-3 days to reduce moisture. For the CHC, coffee husk was used as the plant material but other components (cow dung, wood ash and forest soils) were as for the AWC.

For both systems, four pits were dug with a dimension of 1 m<sup>3</sup>. During the piling of materials for the AWC system, a 5 cm thick layer of maize and wheat straw was placed at the bottom of one pit, followed by up to 20 cm of chopped grasses mixed with cow pea straw and some other green manures on the second layer, then a 15 cm thick layer of cow dung, 5 cm wood ash, and finally a 5 cm layer of forest soil. In between each layer, water was sprinkled to moisten the piles. The same sequence of layers was used until the pit was filled and covered with broad leaved plants and grasses. In the CHC system, the first 25-30 cm layer was covered by the coffee husk, followed by 15 cm cow dung, 5 cm wood ash, and 5 cm forest soil. Similarly, every layer was watered and the sequence was repeated until the pit was filled and finally covered with broad leaves and grasses. The two other pits were left unfilled and used for the compost turning process described below.

The composting process was conducted for a period of 63 days (9 weeks) during which the pit contents were turned and composted alternately between two pits. Morning and afternoon temperature readings of the pits during the composting process were recorded every 2 days (from middle and upper parts of the piles) throughout the 63 days of composting using a 48 inch temperature probe (Composting Thermometer, Reotemp Fast Response, CA, US). On day 21, the substrates in both systems were turned and transferred from the first to the second pit where they were left to compost for another 21 days. On day 42 since compost establishment, they were turned for the second time and transferred back to the first pit for an additional 21 days of composting period up until the termination of the process on the 63<sup>rd</sup> day. In each stage of composting, 10 kg samples were collected, packed and stored in a cold room at 5°C for later extraction of compost teas. In short, three samples

from AWC (AW21, AW42, and AW63) and three samples from CHC (CH21, CH42, and CH63) were available for analysis.

#### *Preparation of non-aerated compost tea*

Samples taken from each stage of composting (AW21, AW42, AW63, CH21, CH42, and CH63) were used for preparation of non-aerated compost teas (NCTs) based on the procedure outlined by Koné *et al.* (2010). Compost and agricultural well water were added to plastic buckets (30 L capacity) in a 1:5 ratio (w/v) to prepare each NCT. The mixture was then allowed to steep for a period of 14 days at room temperature. Layered muslin cloth was used to filter and remove large particles from the respective compost preparations. The NCTs were stored for approximately a week at 4°C until used for DNA extraction and chemical analysis.

#### *Physico-chemical measurements of compost and NCTs*

Physical and chemical analyses of both the parent composts and the compost teas were conducted at the National Soil Testing Laboratories, Addis Ababa. Electrical conductivity (EC) and pH was measured with a pH meter (1: 2.5 H<sub>2</sub>O) (Van Reeuwijk, 1992) for both the parent composts and the compost teas. Total organic carbon of the compost was measured by the Walkley and Black method (Allison *et al.*, 1965) and total nitrogen was determined based on kjeldahl method of nitrogen estimation (Bremner and Mulvaney, 1982). Exchangeable cations and nutrients in both parent compost and NCTs were measured by atomic absorption spectrophotometry (AAS, PG-990, UK).

### *Microbial diversity of the NCTs*

To characterise the microbial communities, genomic DNA was extracted in triplicate from each of the six samples of the NCTs, using the Power Soil DNA kit (MO BIO Laboratories, Inc., USA). A volume of 30 ml of compost tea was centrifuged (2900 x g for 30 min) and up to 0.3 g of the pellets was added to bead-beating tubes from the kit. Subsequently the manufacturer's instructions were followed to purify genomic DNA. DNA quality was measured using a NanoDrop spectrophotometer (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE, U.S.A.).

High throughput amplicon sequencing was conducted according to the methods described by Caporaso *et al.* (2011). Sequencing was performed at MR DNA (Shallowater, TX, USA) using the Illumina MiSeq platform following the manufacturer's guidelines. The V4 variable region of the 16S rRNA gene (for bacteria and archaea) was amplified with the PCR primers 515F/806R and the internal transcribed spacer region (ITS1) of the nuclear ribosomal RNA gene (for fungi) was amplified with primers ITS1-F/ITS2. Pooled PCR products were purified using calibrated Ampure XP beads and used to prepare a DNA library by following the Illumina TruSeq DNA library preparation protocol. Sequence data were processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). Briefly, sequences were joined, depleted of barcodes, denoised, and ambiguous base calls, chimeras and sequence length of <150bp were removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes (DeSantis *et al.*, 2006), RDP II and NCBI ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov), <http://rdp.cme.msu.edu>).

## *Statistical analysis*

The assessment of variation in microbial community composition across NCTs was made using PRIMER 6 (version 6.13) & PERMANOVA+ (version 1.0.3) software packages (PRIMER-E, Plymouth, Ivybridge, United Kingdom). Bray Curtis similarity coefficients were calculated between pairs of samples based on untransformed percentages of the operational taxonomic units (OTUs). Analysis of Similarity (ANOSIM) was used to assess the effect of the stage of composting of NCTs for each compost source on observed bacterial and fungal composition. Principal coordinate analysis by Canonical Analysis of Principal Coordinates (CAP) was used to make ordination diagrams showing the relationship between the structure of the communities (Anderson and Willis, 2003). The relative proportions of bacterial and fungal taxa, at phylum, class and genus levels, were calculated and used to construct tables illustrating the variation in the mean of relative abundance (n=3) of each compost teas sample. Alpha diversity indices (OTU richness and Shannon diversity index H) of both fungal and bacterial communities were determined based on the number of OTUs observed. Significant differences in diversity indices were checked by analysis of variance using the GLM procedure of SAS (SAS Institute, 2011). To determine whether any microbial taxa showed significant differences in abundances between the NCTs of different stages of composting for each compost type, linear discriminant analysis effect size test (Huse *et al.*) was performed according to the procedures described by (Segata *et al.*, 2011) using the online Galaxy tool (<http://huttenhower.sph.harvard.edu/galaxy>). The samples were assigned to six classes: AWCT 21 days, AWCT 42 days AWCT 63 days, CHCT 21 days, CHCT 42 days and CHCT 63 days. Only OTUs that were more than 0.001% of the sequences in that sample were included. The analysis was performed using an alpha value of 0.05 for both the

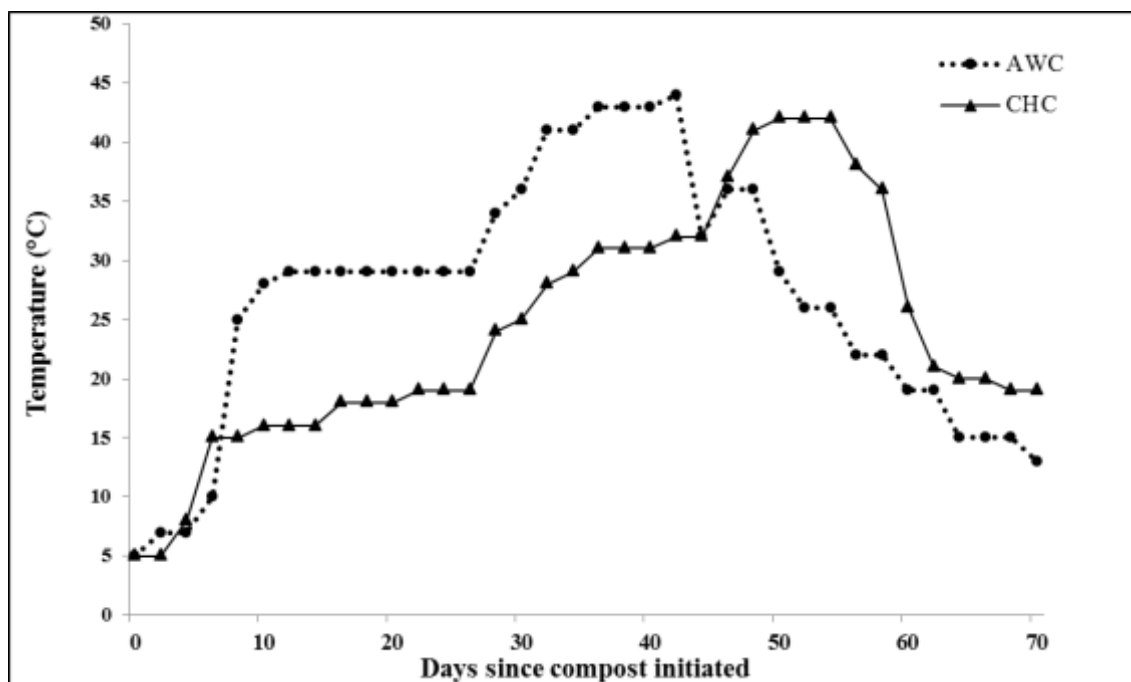


factorial Kruskal-Wallis rank sum test and pairwise Wilcoxon test. Taxa were considered to be differentially abundant if their Linear Discriminant Analysis LDA score was 4 or higher in any given analysis. Linear discriminant analysis effect size was performed to identify taxa that were uniquely abundant in each NCT from particular stages of composting

## 6.4. Results

### *Physicochemical characteristics*

The temporal progression of pit temperature was different for each of the parent composts (Fig. 1). In the first 10 days, the temperature was relatively low in both systems. For AWC, it reached 29°C on day 12 and was then static until the composting pile was transferred to the second pit. At this time, the temperature increased to the maximum of 44°C by day 42 before a steep and relatively steady decline to 19°C by day 63. The pattern of the temperature changes in the CHC, was similar with a generally low temperature in the first week after compost initiation and then a sharp increase to a maximum of 19°C by day 21 followed by a gradual increase in temperature up to the second turning on day 42. At this time, a rise in temperature to >40°C for four consecutive days occurred and was then followed by a steep decline back to 20°C by day 63 (Fig 1).



**Figure 1.** Temperature during the composting process of agricultural waste compost (AWC) and coffee husk compost in a pit composting system. Each temperature was the mean of two measurements.

EC varied with time since compost initiation in both the AWC and CHC (Table 1). The EC for AWCT was lower than that for corresponding parent compost samples 21 and 42 days after compost initiation, whereas the EC for AWCT and the parent compost at 63 days was similar (Table 2). The EC values for CHCT varied with the time of sampling of the parent compost (Table 2). All NCTs and the corresponding parent composts evaluated had neutral pH values (Tables 1 and 2).

The C: N ratio varied with time since compost initiation with the C:N ratio at day 63 being 15.5 and 19.4 for AWC and CHC, respectively (Table 1). There was considerable variation in the amount of Na, K, Ca, and Mg in both types of compost between the 21 and 63 days after

compost initiation (Table 1). Likewise, the extractable cations and the nutrients varied considerably among the NCTs prepared at different stages of composting (Table 2).

### *Amplicon sequencing*

From all the samples of the NCTs, a total of 1,744,302 16S rRNA and 515,658 fungal ITS rRNA gene sequences were obtained by amplicon sequencing after they had been filtered to remove poor quality reads. The number of bacterial gene sequences varied among samples of ACWT, ranging from 72,985 in AWCT63 to 147,622 in AWCT21, with a median of 78,293 for all samples. Likewise, the number of ITS gene sequences ranged from 29,770 in AWC42 to 40,656 in AWCT63, with a median of 32,144 for all samples. The number of bacterial gene sequences varied among samples of CHCT, ranging from 81,290 to 129,689, with a median of 95,928 for all samples. The number of ITS genes ranged from 19,852 to 26,729, with the median of 25,584 for all samples.

**Table 1** Physico-chemical properties of agricultural waste and coffee husk dominated compost sampled from different stages of the composting cycle

Composting cycle <sup>a</sup>	pH H <sub>2</sub> O	EC	OC	TN	C:N	Na	K	Ca	Mg	Fe	Mn	Cu	Zn
	1:2.5	ms/cm	%			Cmol(+)/kg				ppm			
<b>AWC21</b>	7.4	5.8	22.3	1.3	16.6	565	8300	4566	743	8.1	16.9	1.0	24.3
<b>AWC42</b>	7.5	3.5	19.9	1.4	14.2	495	8200	3869	627	11.5	24.4	1.1	18.5
<b>AWC63</b>	7.6	2.8	18.9	1.2	15.5	495	11750	4573	646	13.3	26.9	1.3	18.5
<b>CHC21</b>	7.8	2.3	19.3	1.1	17.2	485	8650	4224	665	8.4	20.3	1.0	30.2
<b>CHC42</b>	7.7	2.0	17.6	0.9	19.1	470	6350	4042	635	7.7	24.7	0.8	27.6
<b>CHC63</b>	7.8	2.0	16.5	0.8	19.3	480	6200	4483	655	6.9	26.9	0.8	30.9

<sup>a</sup> Composting cycle is the different stages of the pit composting system for two types of organic wastes (AWC-Agricultural wastes, CHC-Coffee husk containing waste) piled in equal proportion in pit: AWC21 & CHC21 —samples taken at 21<sup>st</sup> day in pit #1, AWC42 & CHC42 — samples taken at 42<sup>nd</sup> day in pit #2, AWC63& CHC63 — samples taken at 63<sup>rd</sup> day in pit #1. OC = organic carbon and TN = total nitrogen.

**Table 2.** Physico-chemical properties of the nonaerated compost teas made from the different stages of agricultural waste and coffee husk composting substrates. The nutrient concentrations are ppm.

<b>NCTs<sup>b</sup></b>	<b>p<sup>H</sup> H<sub>2</sub>O</b>	<b>EC</b>	<b>Na</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>Fe</b>	<b>Mn</b>	<b>Cu</b>	<b>Zn</b>
	1:2.5	ms/cm								
<b>AWCT21</b>	7.3	2.1	180	1230	6.1	43.4	9.2	9.2	0.06	0.28
<b>AWCT42</b>	7.3	1.7	245	1020	8.9	43.3	7.4	8.1	0.07	0.2
<b>AWCT63</b>	7.3	2.1	315	1505	6.3	55.6	3.9	18.5	0.11	0.25
<b>CHCT21</b>	7.2	2.2	330	1685	11.2	63.1	4.2	26.9	0.15	0.33
<b>CHCT42</b>	7.2	1.9	340	1340	4.5	52.1	10.1	6.9	0.1	0.05
<b>CHCT63</b>	7.3	1.4	325	690	3.9	38.7	6.9	4.9	0.08	0.2

<sup>b</sup> Non-aerated compost teas: compost teas were extracted in a ratio of 1:5 (w/v) by agricultural well water for a period of 14 days. The parent composts from 21 and 42 days were stored at 5–8°C after sampling and NCT prepared at the final date of sampling (63 days).

## Microbial diversity and community structure

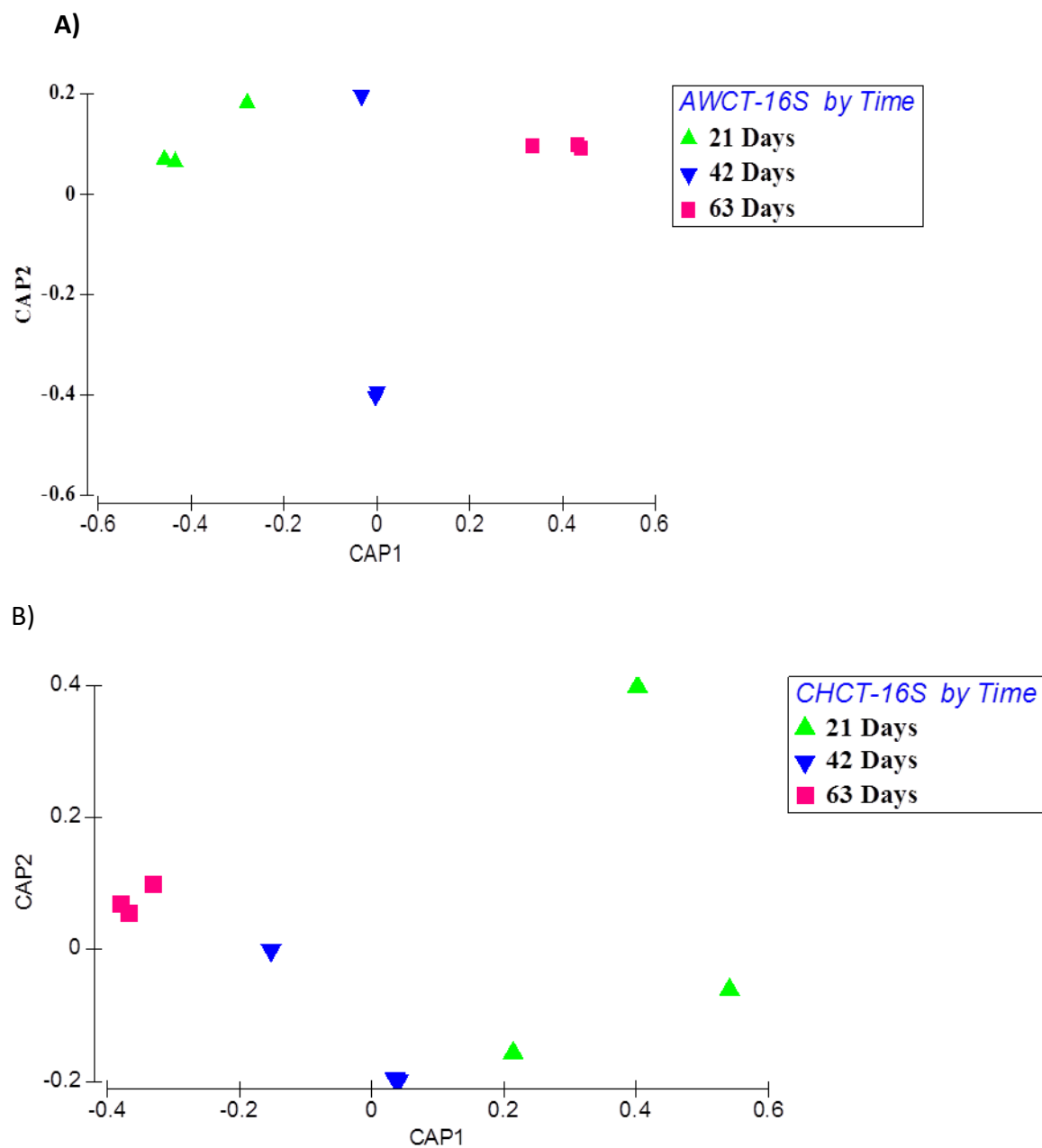
Analysis of differences in the NCT microbial communities across the composting stages were independently computed for the two sources of compost. The overall bacterial community structure of the three composting stages in both AWCT and CHCT types were significantly different according to ANOSIM (global test  $R$ : 0.687,  $p=0.011$  and global test  $R$ : 0.424,  $p=0.007$ , respectively). Similarly, ANOSIM showed a significant difference between overall fungal community patterns among the three composting stages of AWCT (global  $R$ : 0.51,  $p=0.011$ ), but not in CHCT (global  $R$ : 0.25,  $P=0.075$ ). Community structures were significantly different between some of the stages of composting when compared by pair-wise similarity analysis but no significant differences were observed between others (Table 3).

**Table 3.** Results of pair-wise similarity analysis of bacterial and fungal community structures in compost tea prepared from compost at different times after compost initiation. AWCT = agricultural waste compost tea; CHCT = coffee husk compost tea.

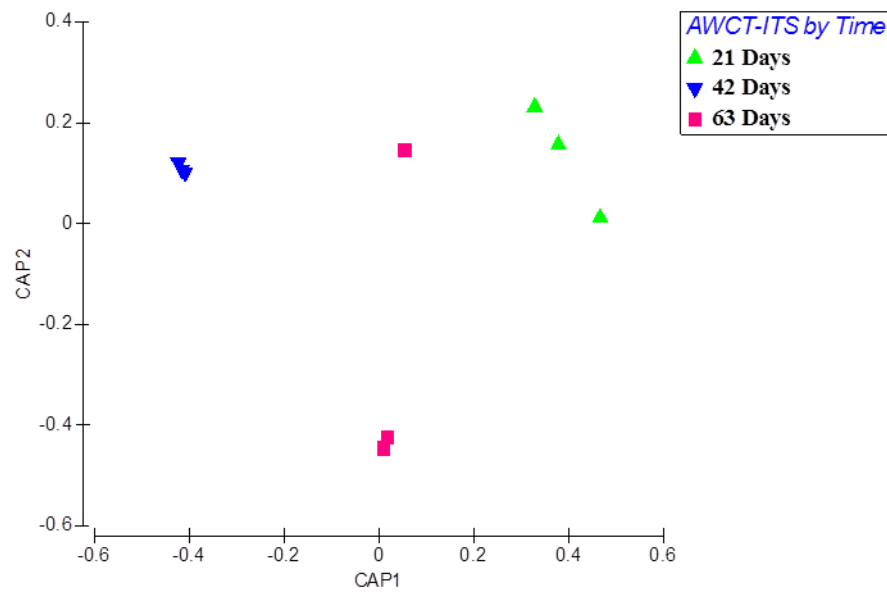
Pair-wise similarity tests	$R$ statistic	$P$ value of significance*
<u>AWCT-bacterial communities</u>		
AWCT21, AWCT42	0.333	0.2
<b>AWCT21, AWCT63</b>	<b>1</b>	<b>0.1</b>
AWCT42, AWCT63	0.556	0.2
<u>CHCT-bacterial communities</u>		
CHCT21, CHCT42	0.2	0.2
CHCT21, CHCT63	0.3	0.1
<b>CHCT42, CHCT63</b>	<b>0.9</b>	<b>0.1</b>
<u>AWCT-fungal communities</u>		
AWCT21, AWCT42	0.4	0.1
AWCT21, AWCT63	0.5	0.2
<b>AWCT42, AWCT63`</b>	<b>0.6</b>	<b>0.1</b>
<u>CHCT-fungal communities</u>		
<b>CHCT21, CHCT42</b>	<b>0.5</b>	<b>0.1</b>
CHCT21, CHCT63	0.1	0.4
CHCT42, CHCT63	0.4	0.1

\*values in bold indicate a significant difference in community structure based on ANOSIM pair-wise similarity analysis. Results are considered significant if  $P = 0.1$  and  $R > 0.5$

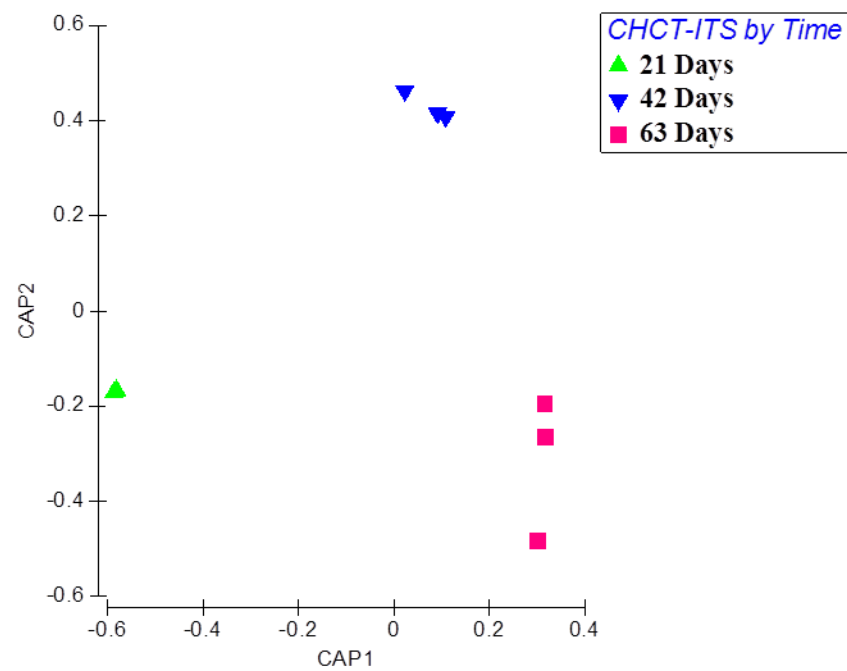
CAP analysis of the bacterial community structure for both AWCT (Fig 2A) and CHCT (Fig 2B) showed that the samples from day 42 were intermediate to the samples from day 21 and day 63. For the fungal communities, the CAP analysis revealed distinctly and equally different communities were present at days 21, 42 and 63 in both the AWCT (Fig 2C) and CHCT (Fig 2D).



C)



D)



**Figure 2 (A-D)** Canonical analyses of principal coordinates (CAP) for bacterial communities in agricultural waste compost tea (A) and coffee husk compost tea (B) and fungal communities in agricultural waste compost tea (C) and coffee husk compost tea (D). Each NCT was produced from one of three different stages of composting (21 days-green, 42 days -blue and 63 days-pink)



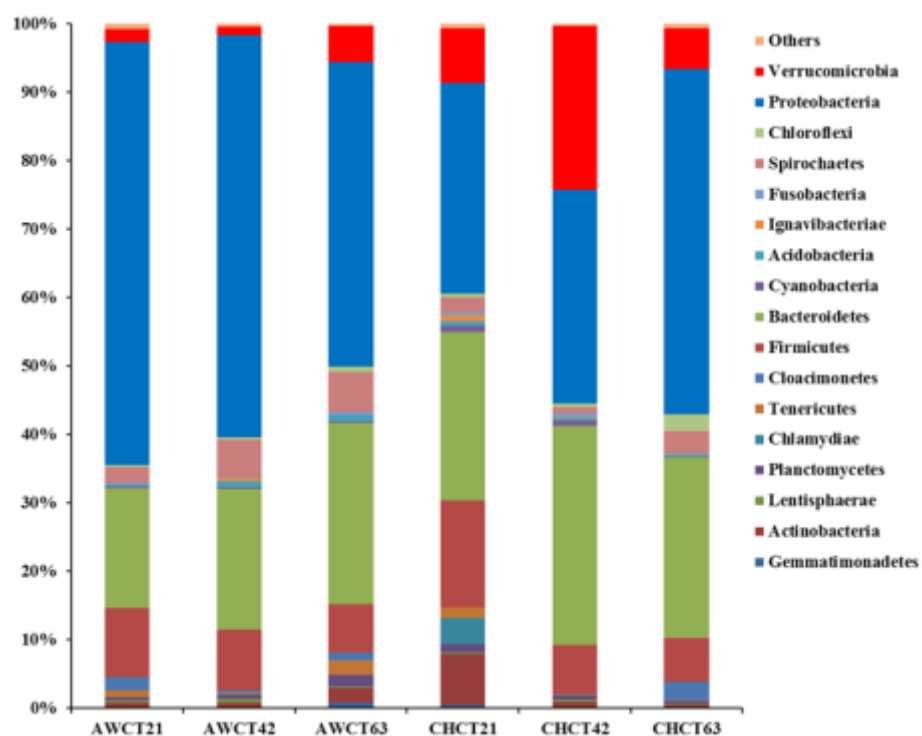
### *Variation in community compositions*

The most abundant bacterial phyla identified from both NCT sources are presented in Fig 3A. There were differences in the mean relative abundances of phyla 21, 42 and 63 days after compost initiation. The most abundant phyla were *Proteobacteria* in AWCT (61% at 21 days, 59% at 42 days, and 44% at 63 days) and in CHCT (30%, 31% and 50%), *Bacteroidetes* in AWCT (17%, 20% and 26%) and in CHCT (24%, 32% and 26%), *Firmicutes* in AWCT (10%, 8% and 7%) and in CHCT (15%, 7%, and 6%), *Verrucomicrobia* in AWCT (1.9%, 1.2% and 5% ) and in CHCT (8%, 23% and 6%), and *Actinobacteria* in AWCT (0.7%, 0.7% to 2%) and in CHCT (7%, 0.8%, and 0.4%).

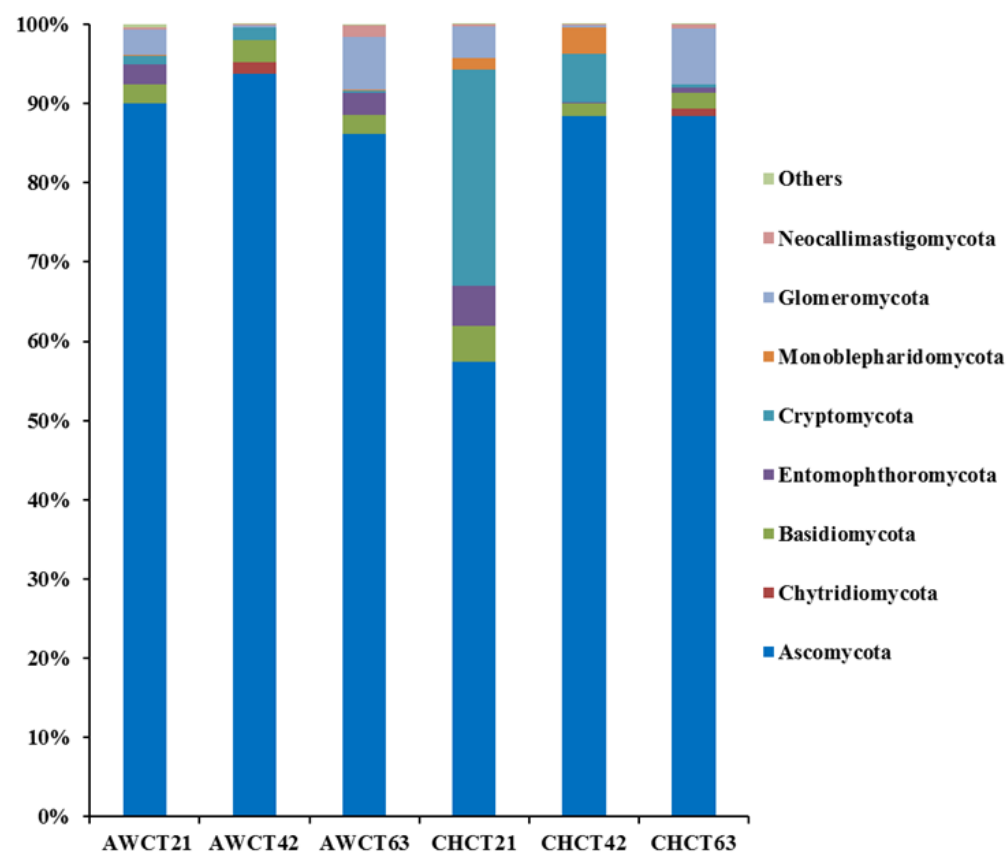
Mean relative abundances of fungal communities at phylum level are presented in Fig 3B.

The most abundant phyla were *Ascomycota* in AWCT (90% at 21 days, 94% at 42 days, and 86% at 63 days) and in CHCT (57%, 86%, and 88%), *Basidiomycota* in AWCT (2%, 3%, and 2%) and in CHCT (4%, 2%, and 2%), *Entomophthoromycota* in AWCT (3%, 0.01%, and 3%) and in CHCT (5%, 0.1%, and 1%), *Cryptomycota* in AWCT (1.1%, 2%, and 0.2%) and in CHCT (27%, 6%, and 0.3%), and *Glomeromycota* in AWCT (3%, 0.2%, and 7%) and in CHCT (4%, 0.3%, and 7%).

A)



B.



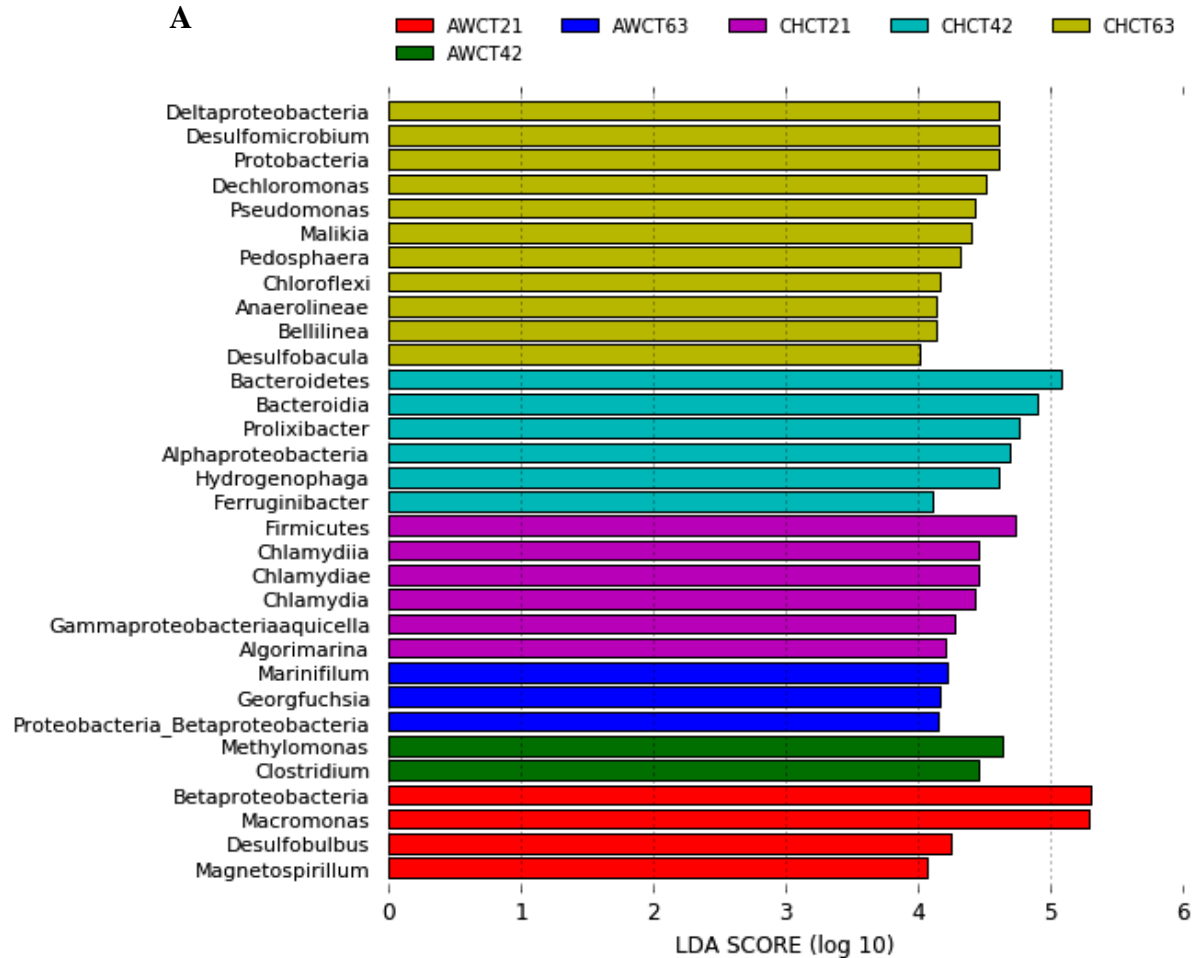
**Figure 3.** Relative abundances expressed as percentage of the total number of sequences of the major bacterial (A) and fungal (B) phyla in both compost teas: AWCT (Agricultural waste compost tea) & CHCT (Coffee husk compost tea)

Diverse bacterial and fungal genera were identified in each type of NCT and their relative abundances varied among NCTs prepared from composts sampled at the different times after compost initiation (Tables S1 and S2).

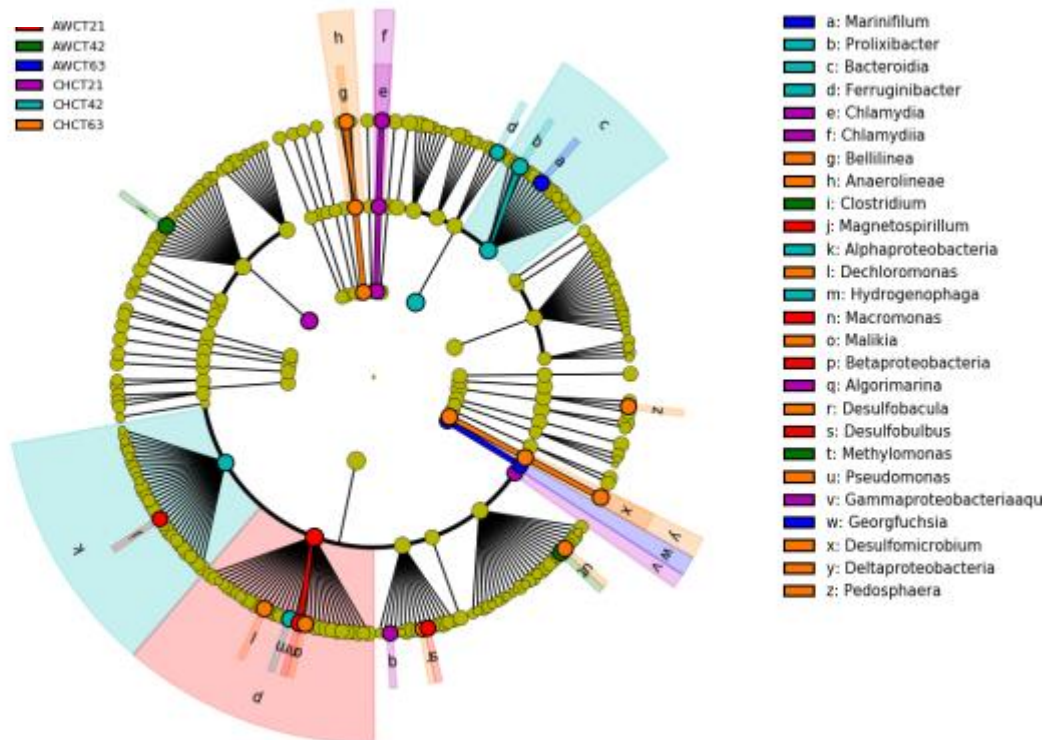
In the AWCT, only a few bacterial taxa were differentially abundant for the three composting stages. Most of these taxa belonged to the phylum Proteobacteria which was the dominant phylum (Fig 3A). The genus *Macromonas* was the most differentially abundant at day 21 with an LDA score over 5 (Fig 4A). There were more discriminatory taxa for the three stages of composting for the CHCT. Genera from the phyla Firmicutes, Chlamydia and the class Gammaproteobacteria were more abundant at day 21, Bacteroidetes and Alphaproteobacteria were more abundant at day 42 and the phyla Verrucomicrobia and Chloroflexi along with classes Alphaproteobacteria and Deltaproteobacteria were more abundant at day 63 (Fig 4A). The circular cladogram of LEfSe results identified bacterial OTUs showing statistical differences between the NCTs distributed according to phylogenetic characteristics and indicates that many OTUs that showed similar abundance in all NCTs (Figure 4B).

Similarly, specific fungal genera were detected to differentially dominate in abundance for different stages of composting and thus in the NCTs (Fig 4C). Fungal clades in the phylum Ascomycota were dominant communities in all the AWCT and the CHCT21 and CHCT42. CHCT63 contained fungal clade represented by the genus *Archaeospora* and phylum Mucoromycota as statistically and biologically abundant taxa (Fig 4C).

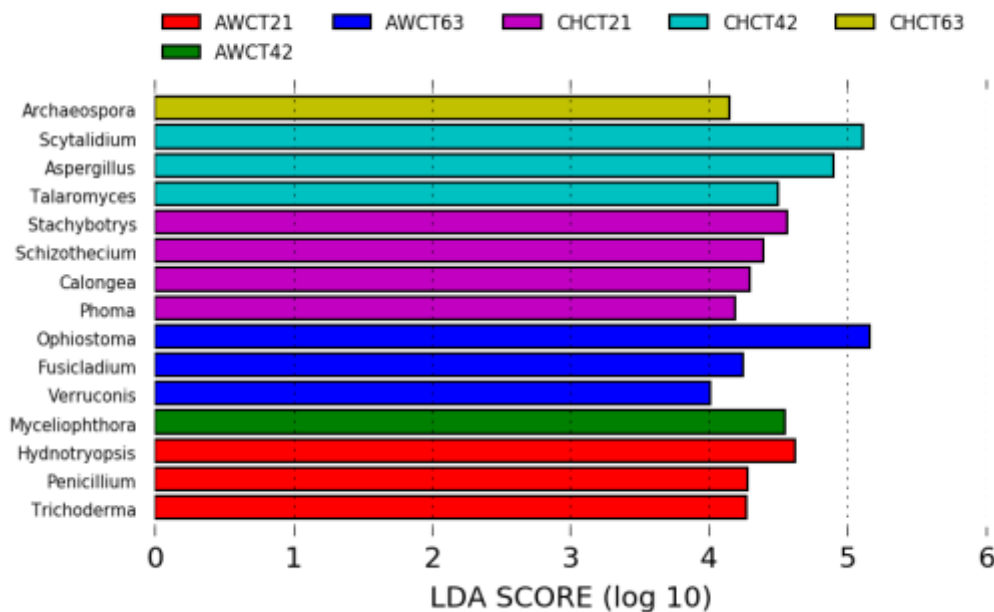
A



B



C



**Figure 4 (a-c).** LEfSe results on microbial communities among non-aerated compost teas (NCTs) from two pit-composting systems-agricultural waste tea (AWCT) and coffee husk based tea (CHCT) each at three stages of composting (21, 42 or 63 days after compost

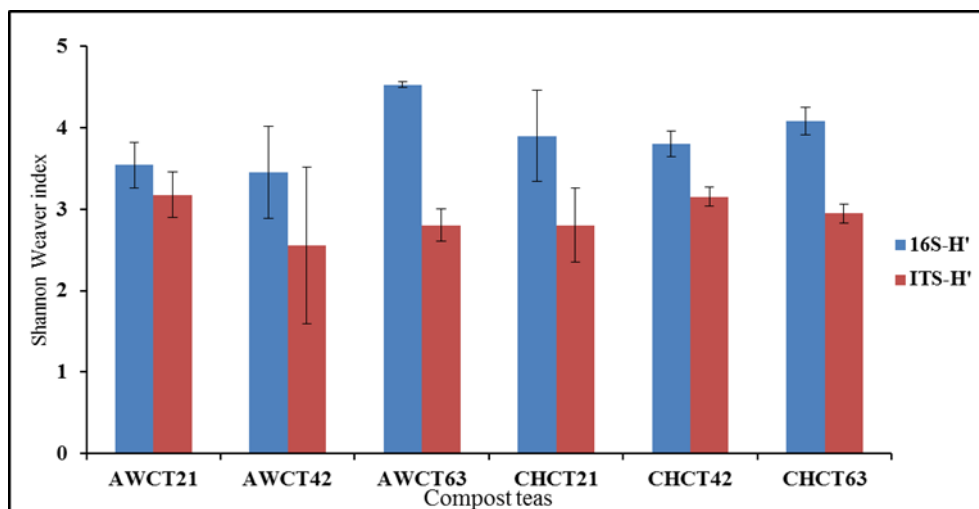
initiation). (a) Histogram of the linear discriminant analysis scores computed for bacterial taxa at phylum, class, and genus levels that were differentially abundant between NCTs; (b) Circular cladogram of LEfSe results identified bacterial OTUs showing statistical differences between the NCTs distributed according to phylogenetic characteristics, the small circles are OTUs and the colour and the sectors indicate the NCTs in which the respective taxa were abundant; yellow colour circles indicates OTUs that showed similar abundance in all NCTs; (c) Histogram of the linear discriminant analysis scores computed for fungal taxa at phylum, class, and genus levels that were differentially abundant between NCTs.

### *Community diversity*

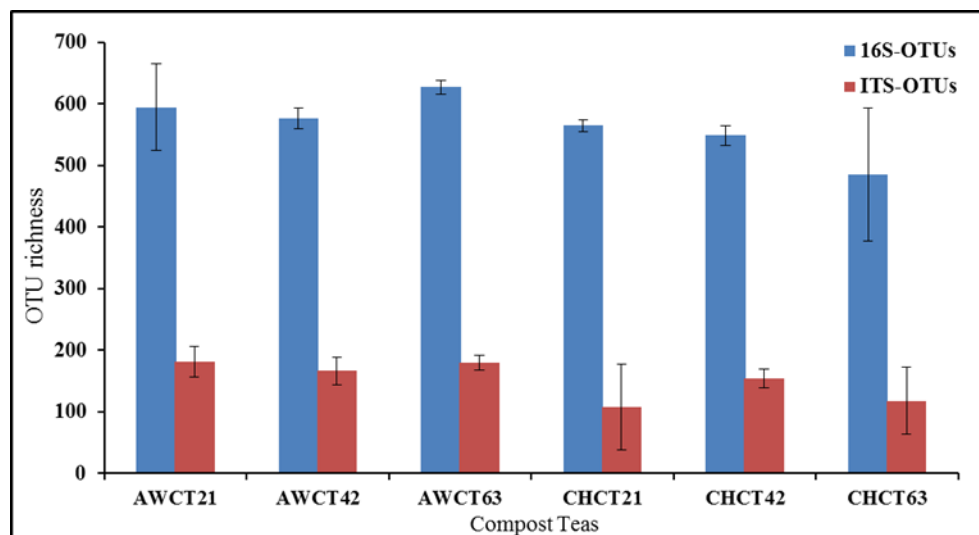
Microbial diversity as measured by the Shannon-Weaver index and OTU richness is presented in Fig 5 (A& B). AWCT prepared using compost sampled 63 days after compost initiation had a higher bacterial diversity than AWCTs prepared using compost sampled at 21 or 42 days after compost initiation ( $p=0.02$ ). However, there was no effect of composting stage ( $p=0.38$ ) on bacterial richness of the AWCTs analysed (Fig 5, A & B).

There was no effect of composting stage on the Shannon-weaver diversity ( $p=0.64$ ) or OTU richness ( $p=0.64$ ) of the bacterial communities in the CHCTs (Fig 5, A&B). Similarly, composting stage had no effect, for both compost source types, on Shannon-weaver diversity (AWCTs,  $P=0.5$  & CHCTs,  $P=0.3703$ ) and richness (AWCTs,  $P=0.63$ , CHCTs,  $P=0.55$ ) of the fungal communities.

A)



B)



**Figure 5.** Diversity indices of NCT samples from AWCT (agricultural waste compost tea) (AW) and CHCT (coffee husk compost tea) prepared from compost sampled at different times after compost initiation (21, 42 and 63 days) A) bacterial and fungal Shannon diversity indices (H), B) bacterial and fungal richness in terms of the number of OTUs. Error bar indicates standard error (n=3)

## 6.5. Discussion

This study provides a detailed description of the microbiota in non-aerated compost teas prepared using parent composts from an on-farm pit-composting system in Ethiopia, with composting initiated with known proportions of organic waste substrates. The results of this study complement previous research which has been largely restricted to quantifying the culturable microbial flora during investigations of compost tea production method (Naidu *et al.*, 2010; Scheuerell and Mahaffee, 2004; ). The bacterial and fungal taxa in the NCTs produced in this study were similar to those reported from composting of organic wastes (Neher *et al.*, 2013; ), which is an indication that the steeping of compost in water for the specified duration results in microbial characteristics similar to the parent composts used to prepare the NCTs.

### *Physico-chemical characteristics of parent composts*

Substrate composition, temperature, pH, EC, C:N, moisture, aeration, and the method by which the process is conducted are the major and inter-dependent factors that influence the complex biodynamic process of composting (Gajalakshmi and Abbasi, 2008; Kuo *et al.*, 2004). The pit temperatures in this study were characterised by long mesophilic or very mild thermophilic conditions not exceeding 45°C. In contrast, aerobic and open window composting often reaches a maximum temperature of 70°C by the thermophilic stage. This difference is probably because the low level of available oxygen in an underground anaerobic system could not stimulate a high level of heat-producing microbial activity as an open, aerobic system. Periodic turning to aerate and uniformly mix the substrates in the underground pits hastened the decomposition process. The temperature reached what is



considered the minimum optimum temperature for full decomposition of the organic matter based on reports that the optimum temperature range for microbial activity during composting is 40–70°C (Partanen *et al.*, 2010; Kuo *et al.*, 2004). The results for pH, EC, and C: N values were in the optimum range reported previously for compost with a similar substrate composition and produced to certain quality standards (Adegunloye *et al.*, 2007; Preethu *et al.*, 2007). Similarly, high concentrations of nutrients such as K and Ca that are directly associated with the compost substrates and the micronutrients from mineralization during decomposition shows that pit-based composting can produce nutrient rich compost that can be used for making compost tea from agricultural wastes in a small-scale, on-farm system.

#### *Physico-chemical properties of the compost teas*

The physico-chemical measurements in all samples of NCTs indicated that high concentrations of macro- and micronutrients were extracted when NCT was produced from the parent composts. The NCTs produced from both sources of parent composts contained similar concentrations of extractable cations, pH, EC over the three stages of composting, noting that the storage of compost samples until all were analysed 63 days after compost initiation may have influenced the physico-chemical characteristics of the NCTs. The high concentration of extractable cations such as K and Ca in both types of NCTs was related to the substrate mixture of the parent compost. The addition of wood ash as a liming agent to the composting system, as was done in this study, is known to improve compost quality and enhance the concentration of extractable cations and nutrients (Kuba *et al.*, 2008).

### *Changes in microbial communities*

At all stages of composting for both NCTs, bacterial phyla that were found in high proportions included Proteobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, and Chloroflexi. These taxa are associated with organic matter decomposition and their presence was consistent with previous reports of compost from similar feedstock composition (Green *et al.*, 2004; Ryckeboer *et al.*, 2003b; . ANOSIM analysis showed that composting stage had a key impact on relative abundances of both bacterial and fungal taxa (Fig 3, Table 3, Tables S1 & S2). The succession of organisms during composting is influenced by the self-heating stages such that certain classes of bacteria dominate the composting system or decline sharply just after the thermophilic stage. For instance, Neher *et al.* (2013) reported that both Proteobacteria and Bacteroidetes were abundant following the thermophilic stage but declined as composting progressed and then increased in abundance when the compost was mature. In this study, the fluctuation in temperature of the pit system was for a relatively short period and of lower magnitude than that experienced in aerobic systems. It is a likely that dynamics of the pit composting system may be less well defined (or signposted by internal temperature) than an open, aerobic system. Even so, there were clear differences among the microbial communities of NCTs prepared using compost at different stages. According to Franke-Whittle *et al.* (2014), the short self-heating phase and thus faster decomposition in a controlled, laboratory-based composting facility is dependent on the composition of substrates rather than the method of composting. Based on community structure and composition, the mixture of substrates in this study decomposed fully, despite the fact that the pit-system is less controlled environment than laboratory conditions.

Given an abundance of degradable organic compounds at compost initiation, it was not surprising that genera such as *Macromonas*, *Desulfobulbus*, *Magnetospirillum*, *Methylomonas*, from the different classes of Proteobacteria, were predominant in AWCT prepared using 21 day old compost. This finding at the taxonomic ranks of class and phylum is consistent with a previous report on bacterial assessment during agricultural waste composting by Chandna *et al.* (2013). These genera appeared to have lower abundances at the same stage of composting in the CHCT. However, genera such as *Sphingobacterium* and *Sediminibacterium* from the phylum Bacteroidetes, and *Bacillus* from Firmicutes were highly abundant in CHCT21, perhaps explained by the presence of a high lignocellulose content in coffee husks (Mussatto *et al.*, 2011) and in agreement with previous reports where similar composting substrates were used (Dantur *et al.*, 2015; ).

All stages of composting in both NCT types harboured fungal taxa including Ascomycota, Basidiomycota, Cryptomycota, Entomophthoromycota and Glomeromycota. This result is similar to observations from other studies of the fungi in NCT produced from similar agricultural waste substrates such as crop residues and coffee hull residues mixed with animal manure (De Gannes *et al.*, 2013; Langarica-Fuentes *et al.*, 2014; Ryckeboer *et al.*, 2003b). Detection of Glomeromycota is unexpected given these mycorrhizal fungi are typically obligate plant symbionts, however spores may have survived the compost process and NCT production stages.

We found certain thermophilic/thermotolerant genera in the phylum Ascomycota to markedly vary in abundances between the two sources of compost and with a clear shift between the stages of composting. For example, *Penicillium*, *Aspergillus*, *Scytalidium*, and *Trichoderma* were abundant in the first 21 days and then decreased with time in the NCTs

prepared from agricultural waste. However, they dominated the later stages of composting in the coffee husk based compost while there was a comparatively higher temperature in the system. In both cases, the influence of temperature was clearly observed as a factor in ecological succession of fungal communities. Thermophilic genera are regarded as the most common in composting materials, due to their capacity to degrade a wide range of organic wastes and to thrive in the higher temperatures experienced in the thermophilic stage of composting (Anastasi *et al.*, 2005).

One often-posed concern with compost tea is that pathogenic microbes could proliferate in the finished product and cause plant, animal or human disease. Both human and plant pathogens are normally reduced or killed during the high thermophilic temperature in aerobic composting (Ceustermans *et al.*, 2007; Noble and Roberts, 2004). In this experiment, the potentially pathogenic genera *Clostridium*, *Aspergillus*, and *Penicillium* were detected; however, the methods applied did not distinguish between closely related pathogenic and non-pathogenic microbes. The latter are usually saprophytes colonising decaying organic materials (Langerica-Fuentes *et al.*, 2014).

In summary, both bacterial and fungal communities in NCT were broadly similar to those reported for composts made from similar substrates. This study confirmed that compost tea contains an array of microorganisms and microbial communities that have potential to contribute to soil and plant health (Ahmad *et al.*, 2007; Hadar and Papadopoulou, 2012). Crop producers who use compost and compost teas regularly do so because they perceive there are beneficial effects on plants, including improved soil nutrient cycling and plant nutrition, and plant disease suppression . More research is needed to continue investigation of the effect of compost tea application for improving and/or maintaining soil

and plant health, and for greater understanding of the microbial communities and functional properties associated with beneficial effects.

**Table S1.** The most abundant (top 20% of relative abundance) bacterial genera in the NCTs. Cells shaded red indicate the highest values for relative abundance.

Phylum	Class	Genus	AWCT21	AWCT42	AWCT63	CHCT21	CHCT42	CHCT63
Actinobacteria	Actinobacteria	Corynebacterium	0.03	0.01	0.01	3.12	0.01	0.01
		Bifidobacterium	0.03	0.01	0.01	3.42	0	0.01
Bacteroidetes	Bacteroidia	Anaerophaga	0.37	4.66	0.45	1.03	0.81	0.72
		Bacteroides	1.16	1.78	2.44	2.51	2.08	3.52
	Sphingobacteriia	Sphingobacterium	2.29	1.28	5.56	3.42	2.8	1.27
	Chitinophagia	Sediminibacterium	0.1	0.04	0.05	5.48	0.58	0.95
	Bacteroidia	Prolixibacter	4.02	2.66	2.51	0.42	8.6	0.57
	Flavobacteriia	Flavobacterium	1.36	0.81	2.49	1.51	1.93	4.92
	Chitinophagia	Sediminibacterium	0.1	0.04	0.05	5.48	0.58	0.14
	Opitutae	Opitutus	0.12	0.24	0.96	5.03	0.69	0.19
Verrucomicrobia	Verrucomicrobiae	Brevifollis	0.21	0.03	0.7	0.07	19.11	0.78
		Pedosphaera	0.21	0.83	0.87	0.49	0.64	3.41
Chlamydiae	Chlamydiia	Chlamydia	0.03	0.07	0.01	3.65	0.04	0.02
Firmicutes	Clostridia	Clostridium	4.77	3.89	2.15	1.5	1.44	2.17
		Pelotomaculum	0.18	0.1	0.94	3.53	2.27	0.14
Firmicutes	Bacilli	Bacillus	0.51	0.92	0.35	5.9	0.32	0.24
Proteobacteria	Betaproteobacteria	Dechloromonas	0.92	1.93	0.71	0.58	1.85	5.32
		Malikia	0.33	0.16	0.08	0.15	0.44	4.07
		Hydrogenophaga	1.2	0.52	0.28	0.27	6.42	2.07
		Macromonas	29.76	0.14	0.14	0.09	0.26	0.95
	Gammaproteobacteria	Methylomonas	3.8	3.31	0.02	0.18	0.07	0.05
		Pseudomonas	0.17	0.15	0.3	0.44	0.26	4.1
	Betaproteobacteria	Thiobacillus	0.62	0.77	9.27	0.52	0.94	4.93
	Deltaproteobacteria	Candidatus babelia	0.03	0.01	0.01	3.27	0.01	0.02
		Desulfobulbus	3.08	0.59	0.33	0.07	0.5	2.25

Geobacter	3.33	8.51	0.87	0.32	0.97	1.47
Desulfomicrobium	0.69	0.97	2.85	0.34	0.87	6.32

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**Table S2.** The most abundant fungal taxa. Cells shaded red indicate the highest values for relative abundance.

Phylum	Class	Genus	AWCT21	AWCT42	AWCT63	CHCT21	CHCT42	CHCT63
Ascomycota	Dothideomycetes	Fusicladium	89.968	93.768	86.149	57.425	88.440	88.397
			1.02	0.62	6.21	2.70	0.37	1.81
	Eurotiomycetes	Penicillium	0.741	0.022	4.240	0.155	0.078	1.689
			23.25	38.57	13.46	6.89	32.91	13.99
			3.661	0.660	0.163	0.168	1.108	0.573
			0.211	0.280	0.081	0.206	3.252	0.220
	Leotiomyces	Aspergillus	7.015	27.687	6.912	0.180	7.822	7.321
			11.589	8.524	4.002	4.229	19.710	3.442
			21.19	16.91	7.47	0.76	26.95	28.95
			21.171	16.909	7.467	0.756	26.951	28.932
	Pezizomycetes	Calongea	12.70	1.15	11.25	10.92	0.64	2.37
			3.315	0.037	3.048	6.003	0.132	0.880
			8.465	0.578	4.853	0.359	0.078	0.074
			0.006	0.013	0.011	3.378	0.007	0.007
	Sordariomycetes	Terfezia	30.52	35.96	47.08	34.63	26.79	40.10
			2.921	5.333	1.467	4.677	4.779	9.012
			0.064	2.082	0.075	2.946	0.559	0.937
			2.286	8.074	1.697	0.810	7.355	1.441
			8.812	0.265	34.262	2.230	0.849	14.929
			0.304	3.802	0.203	5.343	3.623	3.516
			3.342	1.363	0.034	1.334	0.047	0.067
			5.549	1.847	1.990	1.341	0.778	1.797
Basidiomycota	Tremellomycetes	Trichosporon	2.425	2.828	2.376	4.445	1.558	2.012
			0.16	2.25	0.02	3.54	0.11	0.29
			0.061	2.213	0.014	3.504	0.008	0.259
Chytridiomycota	Monoblepharidomycetes		0.007	1.428	0.010	0.015	0.007	0.890
			0.02	0.09	0.12	1.44	3.29	0.09



		Hyaloraphidium	0.014	0.088	0.117	1.444	3.294	0.091
Cryptomycota			1.146	1.513	0.242	27.272	6.189	0.336
		Paramicrosporidium	1.146	1.513	0.242	27.272	6.189	0.336
Entomophthoromycota			2.507	0.021	2.778	5.129	0.096	0.710
	Basidiobolomycetes		2.51	0.02	2.78	5.13	0.10	0.71
		Basidiobolus	2.507	0.021	2.778	5.129	0.096	0.710
		Others	16.82	18.69	26.35	28.54	13.28	23.77

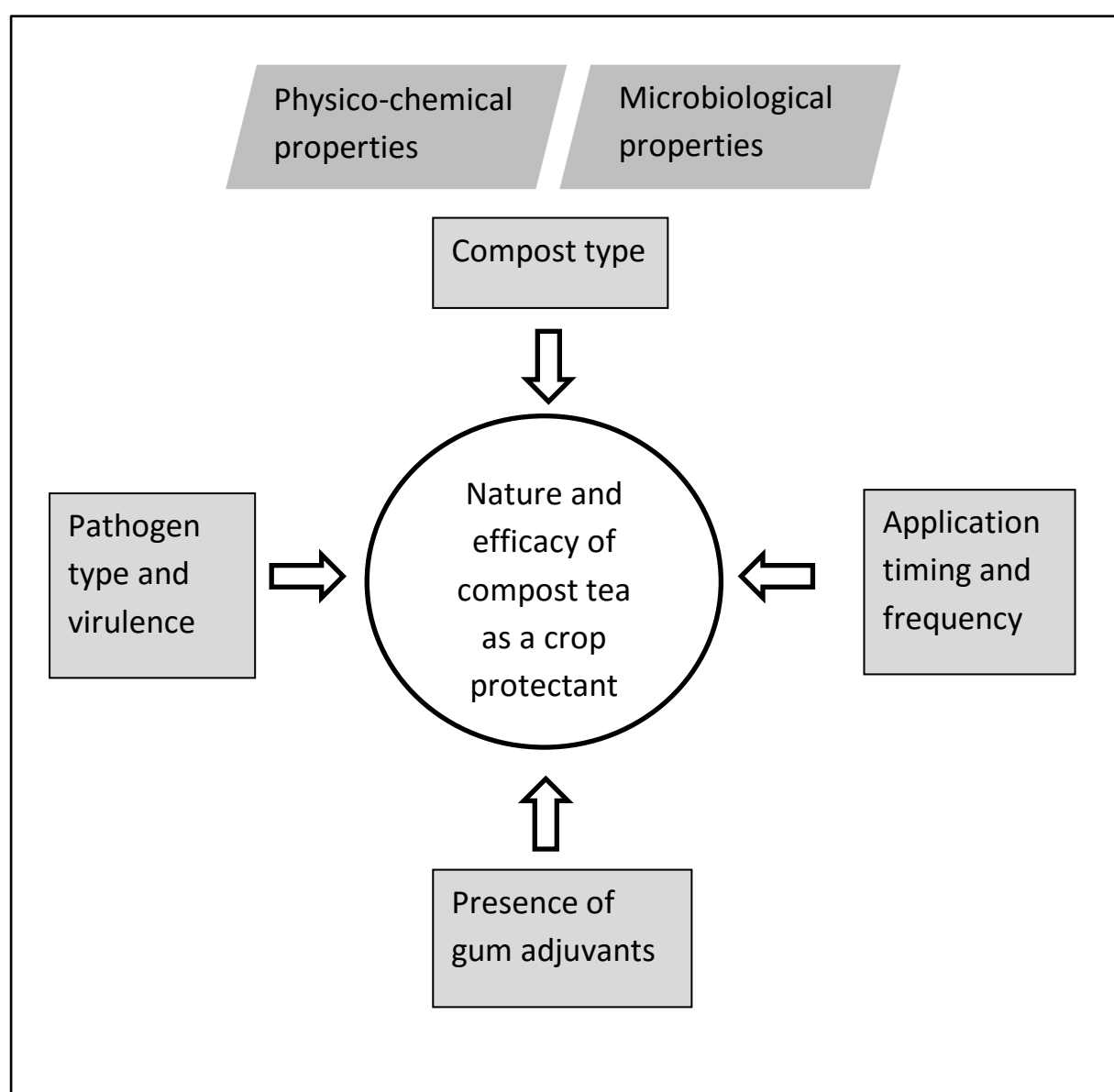
# Chapter 7

## GENERAL DISCUSSION

This thesis describes findings of experiments undertaken to evaluate the suitability of compost extracts as suppressants of key fungal and bacterial diseases of potato. Non-aerated processes were employed to make these compost teas from variable sources for trials conducted in Tasmania, Australia and Ethiopia as reported in experimental chapters 3-6. The non-aerated compost tea method was chosen for its attributes of disease suppressive efficacy on a wide-range of pathosystems and its general simplistic technology and low cost (Brinton *et al.*, 2004; Brinton and Trankner, 1996; ; Litterick *et al.*, 2004).

Foliar and soil borne fungi such as *Rhizoctonia solani*, *Alternaria solani*, and *Alternaria alternata* are ubiquitous potato pathogens that have a huge economic impact on potato production globally and in Tasmanian growing belts in particular, for which fungicides are the main means of control (Horsfield *et al.*, 2010; Sparrow and Wilson, 2012) . Potato is important food commodity and income generating crop in Ethiopia, but suffers from recurrent incidences of soil and foliar pathogens. Bacterial wilt caused by *R. solanacearum* is a disease of high economic importance in potato growing areas. In Tasmania, the organic growers have some option for crop management , whereas in small scale system in Ethiopia, options of crop management are limited. The initiation of these sets of experiments was, therefore, based on the assumptions that alternative management options are relevant for these specific problems.

NCTs evaluated in these studies did provide a variable level of disease suppression, which depended mainly on four major factors: type of parent compost, the pathogen, frequency and timing of application, the presence of carbohydrate gum adjuvants Under the four overarching factors., we investigated the intrinsic characters that define NCTs (microbiological and physico-chemical properties) and propose that these are the key elements that relate to observed NCTs effect in our experimental systems (Figure 1).



**Figure 1.** Major factors and the intrinsic characters (physico-chemical and microbiological) influencing the efficacy of non-aerated compost on the selected diseases of potato in the presented studies.

In this thesis, we presented evidence that the biotic component of NCTs was critical for effective suppression of disease (Chapter 3), however the physico-chemical properties may determine how functional the microbiological agents are. For example, if pH or EC are not in the optimal range, the balance of microbial species is likely to be influenced. Therefore, physico-chemical properties are also critical.

We found that compost type (Chapters 3-6) had a significant effect on these microbial and physico-chemical characters, and importantly on the apparent efficacy of the compost tea to reduce selected potato diseases. We recommend that agricultural waste is the best compost source to use for non-aerated compost tea production for bacterial wilt disease control. This is advantageous for smallholder mixed crop-livestock farmers who have this resource readily available. We suggest that compost age may be less critical than compost type, based on the finding that microbial diversity was similar in compost produced from agricultural waste in open-pit methods and this was reflected in the microbial composition of the NCT (Chapter 6).

The foliar fungal pathogens: *Alternaria alternata* and *Alternaria solani* were both suppressed significantly by the NCTs tested (Chapter 3). These opportunistic pathogens damage potato foliage at stages close to maturity, while synthetic fungicides are sprayed in most cases as protectants. Therefore, spraying compost tea from matured compost made from a mixture of decomposable organic waste could inhibit the incidence of these foliar fungal diseases of potato for organic growers. Even though the study conducted was based on *in vitro* tests using bioassay techniques, these results are important and indicative for further screening and evaluation of NCTs as a foliar spray to control potato pathogens.

In the case of soil borne pathogens, mainly *Ralstonia solanacearum*, we demonstrated that the establishment of the pathogen is significantly reduced when the NCTs of agricultural waste are applied at a time of planting. To further enhance the efficacy, we recommend that application of NCTs should be made frequently (at least once every 7 days) depending on other available resources such as labour and available compost material to frequently make fresh NCTs. This is because of the fact that the intrinsic properties of both the compost and NCTs can change in storage, as was observed in results of stored vermicompost in our study (Chapter 4). The changes in physico-chemical properties and shifts in microbial composition due to addition of compost and other organic amendments to the soil had abilities to reduce the inoculum level and thus resultant wilt disease caused by *R. solanacearum* in previous studies (Fu *et al.*, 2016; Islam *et al.*, 2014; Liu *et al.*, 2015). Therefore, reduction in severity of wilt observed in pot-grown plants due to agricultural waste based NCTs (Chapter 4) does agree with the currently recommended options of bacterial wilt suppression in different pathosystems.

In this thesis, we evaluated the role that additives (mainly carbohydrate gum) play in influencing the efficacy/properties of NCTs tested. Added to the CCT, when used as foliar application against *Alternaria alternata* (Chapter 3), the effect in restricting symptoms was not significantly enhanced compared to the pure CCT application. However, observation of the leaf surface by electron microscopy indicated that addition of gum altered the arrangement and interaction that occurred between the pathogen structure and the microbes from the CCT. The impact of this interaction on actual disease reduction or on the efficacy of the NCT is a remaining area to be investigated further. The application of carbohydrate myrrh however, has enhanced the efficacy of the agricultural waste NCT in the

control of bacterial wilt in our study (Chapter 5). Therefore, it is likely that factors such as the type and purity of gum, its compatibility with the NCT microbes and the pathosystem can play major role in varying the influence of gum adjuvant to NCTs.

This thesis presents evidence and consolidates findings from the current literature that organic waste materials of both urban and rural areas can be converted to compost, from which non-aerated teas can be made which are effective as crop protectants. The compost water extract prepared can provide a meaningful level of crop disease protection, particularly for small-scale to urban farm systems. We recommend that field trials be conducted with potato in Ethiopia to determine how this efficacy is realised in a complex environment in which native soil microorganisms are present. If the benefits of disease control outcomes exceed the cost for small-holder farmers, then practical steps to make and apply NCT need to be demonstrated and familiarized to farmers as part of an integrated disease management system in both conventional and organic farming systems.

## References

- Acero, J. L., Benítez, F. J., Real, F. J. & González, M. (2008). Chlorination of organophosphorus pesticides in natural waters. *Journal of Hazardous Materials* 153(1–2): 320-328.
- Adegunloye, D., Adetuyi, F., Akinyosoye, F. & Doyeni, M. (2007). Microbial analysis of compost using cowdung as booster. *Pakistan Journal of Nutrition* 6(5): 506-510.
- Ahmad, R., Jilani, G., Arshad, M., Zahir, Z. A. & Khalid, A. (2007). Bio-conversion of organic wastes for their recycling in agriculture: an overview of perspectives and prospects. *Annals of microbiology* 57(4): 471-479.
- Al-Dahmani, J. H., Abbasi, P. A., Miller, S. A. & Hoitink, H. A. (2003). Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. *Plant Disease* 87(8): 913-919.
- Alexandratos, N. & Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision. ESA Working paper.
- Aley, E. F. L. G. P. & Elphinstone, J. (1995). Culture media for *Ralstonia solanacearum* isolation, identification and maintenance. *Fitopatologia* 30: 126-130.
- Alfano, G., Lustrato, G., Lima, G., Vitullo, D. & Ranalli, G. (2011). Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. *Biological Control* 58(3): 199-207.
- Allison, L., Bollen, W. & Moodie, C. (1965). Total carbon. *Methods of soil analysis. Part 2. Chemical and microbiological properties* (methods of soil analysis): 1346-1366.
- Álvarez, B., Biosca, E. G. & López, M. M. (2010a). On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. *Current research, technology and education topics in applied microbiology and microbial biotechnology* 1: 267-279.
- Álvarez, B., Biosca, E. G. & López, M. M. (2010b). On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. *Current research, technology and education topics in applied microbiology and microbial biotechnology. Volume 1*.
- Álvarez, B., Vasse, J., Le-Courtois, V., Trigalet-Démery, D., López, M. & Trigalet, A. (2008). Comparative behavior of *Ralstonia solanacearum* biovar 2 in diverse plant species. *Phytopathology* 98(1): 59-68.
- Anastasi, A., Varese, G. C. & Marchisio, V. F. (2005). Isolation and identification of fungal communities in compost and vermicompost. *Mycologia* 97(1): 33-44.
- Anderson, M. J. & Willis, T. J. (2003). Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84(2): 511-525.
- Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R. & Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution* 19(10): 535-544.
- Andrews, J. & Harris, R. (1992). Compost extracts and the biological control of apple scab. *Can. J. Plant Pathol* 14: 240.
- Anith, K. N., Momol, M. T., Kloepper, J. W., Marois, J. J., Olson, S. M. & Jones, J. B. (2004). Efficacy of Plant Growth-Promoting Rhizobacteria, Acibenzolar-S-Methyl, and Soil Amendment for Integrated Management of Bacterial Wilt on Tomato. *Plant Disease* 88(6): 669-673.
- Arora, R. & Khurana, S. P. (2004). Major fungal and bacterial diseases of potato and their management. In *Fruit and vegetable diseases*, 189-231: Springer.
- Arrebola, E., Sivakumar, D., Bacigalupo, R. & Korsten, L. (2010). Combined application of antagonist *Bacillus amyloliquefaciens* and essential oils for the control of peach postharvest diseases. *Crop Protection* 29(4): 369-377.

- Aryantha, I. P., Cross, R. & Guest, D. I. (2000). Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. *Phytopathology* 90(7): 775-782.
- Bais, H. P., Fall, R. & Vivanco, J. M. (2004). Biocontrol of *Bacillus subtilis* against Infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiology* 134(1): 307-319.
- Beauregard, P. B., Chai, Y., Vlamakis, H., Losick, R. & Kolter, R. (2013). *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proceedings of the National Academy of Sciences* 110(17): E1621-E1630.
- Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* 84(1): 11-18.
- Berg, G., Opelt, K., Zachow, C., Lottmann, J., Götz, M., Costa, R. & Smalla, K. (2006). The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. *FEMS Microbiology Ecology* 56(2): 250-261.
- Bill, M., Sivakumar, D., Korsten, L. & Thompson, A. K. (2014). The efficacy of combined application of edible coatings and thyme oil in inducing resistance components in avocado (*Persea americana* Mill.) against anthracnose during post-harvest storage. *Crop Protection* 64: 159-167.
- Bogino, P. C., Oliva, M. d. I. M., Sorroche, F. G. & Giordano, W. (2013). The role of bacterial biofilms and surface components in plant-bacterial associations. *International journal of molecular sciences* 14(8): 15838-15859.
- Boulter, J. I., Trevors, J. T. & Boland, G. J. (2002). Microbial studies of compost: bacterial identification, and their potential for turfgrass pathogen suppression. *World Journal of Microbiology and Biotechnology* 18(7): 661-671.
- Bremner, J. M. & Mulvaney, C. (1982). Nitrogen—total. *Methods of soil analysis. Part 2. Chemical and microbiological properties* (methodsofsoilan2): 595-624.
- Brewer, L. J. & Sullivan, D. M. (2003). Maturity and Stability Evaluation of Composted Yard Trimmings. *Compost Science & Utilization* 11(2): 96-112.
- Brinton, W., Storms, P., Evans, E. & Hill, J. (2004). Compost teas: microbial hygiene and quality in relation to method of preparation. *Biodynamics*: 36-45.
- Brinton, W. F. & Droffner, M. (1995). The control of plant pathogenic fungi by use of compost teas. *Biodynamics* 197: 12-15.
- Brinton, W. F. & Trankner, A. (1996). Investigations into liquid compost extracts. *Biocycle* 37(11): 68.
- Burges, H. D. (2012). *Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments*. Springer Science & Business Media.
- Campbell, C. L. & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. John Wiley & Sons.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L. & Bauer, M. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal* 6(8): 1621-1624.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N. & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* 108(Supplement 1): 4516-4522.
- Ceustermans, A., De Clercq, D., Aertsen, A., Michiels, C., Coosemans, J. & Ryckeboer, J. (2007). Inactivation of *Salmonella* Senftenberg strain W 775 during composting of biowastes and garden wastes. *Journal of Applied Microbiology* 103(1): 53-64.
- Champoiseau, P. G., Jones, J. B. & Allen, C. (2009). *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. *Plant Health Progress* 10: 1-10.



- Chandna, P., Nain, L., Singh, S. & Kuhad, R. C. (2013). Assessment of bacterial diversity during composting of agricultural byproducts. *BMC Microbiology* 13: 99-99.
- Chen, K.-N., Chen, C.-Y., Lin, Y.-C. & Chen, M.-J. (2013a). Formulation of a novel antagonistic bacterium based biopesticide for fungal disease control using microencapsulation techniques. *Journal of Agricultural Science* 5(3): 153.
- Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R. & Guo, J.-h. (2013b). Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environmental Microbiology* 15(3): 848-864.
- Collins, D. P., Jacobsen, B. J. & Maxwell, B. (2003). Spatial and temporal population dynamics of a phyllosphere colonizing *Bacillus subtilis* biological control agent of sugar beet cercospora leaf spot. *Biological Control* 26(3): 224-232.
- Crane, J. M. & Bergstrom, G. C. (2014). Spatial distribution and antifungal interactions of a *Bacillus* biological control agent on wheat surfaces. *Biological Control* 78(0): 23-32.
- Cronin, M. J., Yohalem, D. S., Harris, R. F. & Andrews, J. H. (1996). Putative mechanism and dynamics of inhibition of the apple scab pathogen *Venturia inaequalis* by compost extracts. *Soil Biology and Biochemistry* 28(9): 1241-1249.
- Danhorn, T. & Fuqua, C. (2007). Biofilm formation by plant-associated bacteria. *Annual Review of Microbiology* 61: 401-422.
- Dannon, E. A. & Wydra, K. (2004). Interaction between silicon amendment, bacterial wilt development and phenotype of *Ralstonia solanacearum* in tomato genotypes. *Physiological and Molecular Plant Pathology* 64(5): 233-243.
- Danon, M., Franke-Whittle, I. H., Insam, H., Chen, Y. & Hadar, Y. (2008). Molecular analysis of bacterial community succession during prolonged compost curing. *FEMS Microbiology Ecology* 65(1): 133-144.
- Danon, M., Zmora-Nahum, S., Chen, Y. & Hadar, Y. (2007). Prolonged compost curing reduces suppression of *Sclerotium rolfsii*. *Soil Biology and Biochemistry* 39(8): 1936-1946.
- Dantur, K. I., Enrique, R., Welin, B. & Castagnaro, A. P. (2015). Isolation of cellulolytic bacteria from the intestine of *Diatraea saccharalis* larvae and evaluation of their capacity to degrade sugarcane biomass. *AMB Express* 5: 15.
- De Gannes, V., Eudoxie, G. & Hickey, W. (2013). Insights into fungal communities in composts revealed by 454-pyrosequencing: implications for human health and safety. *Frontiers in Microbiology* 4(164).
- Deepthi, P. & Reddy, P. N. (2013). Compost Teas—An organic source for crop disease management. *International Journal of Innovative Biological Research* 2(1): 51-60.
- DeFauw, S. L., He, Z., Larkin, R. P. & Mansour, S. A. (2012). Sustainable potato production and global food security. In *Sustainable potato production: global case studies*, 3-19: Springer.
- DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P. & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72(7): 5069-5072.
- Dey, R., Pal, K. K. & Tilak, K. (2014). Plant Growth Promoting Rhizobacteria in Crop Protection and Challenges. In *Future Challenges in Crop Protection Against Fungal Pathogens*, 31-58: Springer.
- Diénez, F., Santos, M., Boix, A., De Cara, M., Trillas, I., Avilés, M. & Tello, J. (2006). Grape marc compost tea suppressiveness to plant pathogenic fungi: role of siderophores. *Compost Science & Utilization* 14(1): 48-53.
- Ding, C., Shen, Q., Zhang, R. & Chen, W. (2013). Evaluation of rhizosphere bacteria and derived bio-organic fertilizers as potential biocontrol agents against bacterial wilt (*Ralstonia solanacearum*) of potato. *Plant and Soil* 366(1-2): 453-466.
- Dionne, A., Tweddell, R. J., Antoun, H. & Avis, T. J. (2012). Effect of non-aerated compost teas on damping-off pathogens of tomato. *Canadian Journal of Plant Pathology* 34(1): 51-57.

- Edwards, C. A., Dominguez, J. & Arancon, N. Q. (2004). The influence of vermicompost on plant growth and pest incidence. *Soil Zoology for Sustainable Development in the 21st Century, Cairo*: 397-420.
- El-Haddad, M. E., Zayed, M. S., El-Sayed, G. A. M., Hassanein, M. K. & Abd El-Satar, A. M. (2014). Evaluation of compost, vermicompost and their teas produced from rice straw as affected by addition of different supplements. *Annals of Agricultural Sciences* 59(2): 243-251.
- El-Masry, M., Khalil, A., Hassouna, M. & Ibrahim, H. (2002). In situ and in vitro suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. *World Journal of Microbiology and Biotechnology* 18(6): 551-558.
- El Ghaouth, A., Wilson, C. & Wisniewski, M. (2004). Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases of fruit and vegetables. In *Diseases of Fruits and Vegetables: Volume II*, 511-535: Springer.
- Evans, K., Palmer, A. & Metcalf, D. (2013). Effect of aerated compost tea on grapevine powdery mildew, botrytis bunch rot and microbial abundance on leaves. *European Journal of Plant Pathology* 135(4): 661-673.
- Evans, K. J. & Percy, A. K. (2014). Integrating Compost Teas in the Management of Fruit and Foliar Diseases for Sustainable Crop Yield and Quality. In *Composting for Sustainable Agriculture*, 173-198 (Ed D. K. Maheshwari). Springer.
- FAO (1980). A manual of rural composting. FAO/UNDP Regional Project RAS/75/004 Field Document No. 15. Rome.
- FAO (2008). Potato World—International Year of the Potato/<http://www.potato2008.org/en/world/africa.html>.
- FAO, U. (2009). How to Feed the World in 2050. In *Rome: High-Level Expert Forum*.
- FAO/WHO (1999). Compendium of food additive specifications ( Addendum 7)/Joint FAO/WHO Expert Committee on Food Additives.
- Fiers, M., Edel-Hermann, V., Chatot, C., Le Hingrat, Y., Alabouvette, C. & Steinberg, C. (2012). Potato soil borne diseases. A review. *Agronomy for Sustainable Development* 32(1): 93-132.
- Franke-Whittle, I. H., Confalonieri, A., Insam, H., Schlegelmilch, M. & Körner, I. (2014). Changes in the microbial communities during co-composting of digestates(). *Waste Management (New York, N.y.)* 34(3): 632-641.
- Fu, L., Ruan, Y., Tao, C., Li, R. & Shen, Q. (2016). Continuous application of bioorganic fertilizer induced resilient culturable bacteria community associated with banana Fusarium wilt suppression. *Scientific Reports* 6: 27731.
- Gado, E. (2013). Induction of Resistance in Potato Plants against Bacterial Wilt Disease under Egyptian Condition. *Journal of Applied Sciences Research* 9(1): 170-177.
- Gajalakshmi, S. & Abbasi, S. A. (2008). Solid Waste Management by Composting: State of the Art. *Critical Reviews in Environmental Science and Technology* 38(5): 311-400.
- Gamliel, A., Austerweil, M. & Kritzman, G. (2000). Non-chemical approach to soil borne pest management – organic amendments. *Crop Protection* 19(8–10): 847-853.
- Gao, M., Liang, F., Yu, A., Li, B. & Yang, L. (2010). Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. *Chemosphere* 78(5): 614-619.
- Gea, F., Navarro, M. & Tello, J. (2009). Potential application of compost teas of agricultural wastes in the control of the mushroom pathogen *Verticillium fungicola*. *Journal of Plant Diseases and Protection* 116(6): 271-273.
- Gebrehiwot, M., Asres, K., Bisrat, D., Mazumder, A., Lindemann, P. & Bucar, F. (2015). Evaluation of the wound healing property of *Commiphora guidottii* Chiov. ex. Guid. *BMC Complementary and Alternative Medicine* 15(1): 1-11.
- Gibson, S. & Kurilich, A. (2013). The nutritional value of potatoes and potato products in the UK diet. *Nutrition Bulletin* 38(4): 389-399.

- Gildemacher, P. R., Kaguongo, W., Ortiz, O., Tesfaye, A., Woldegiorgis, G., Wagoire, W. W., Kakuhenzire, R., Kinyae, P. M., Nyongesa, M., Struik, P. C. & Leeuwis, C. (2009). Improving Potato Production in Kenya, Uganda and Ethiopia: A System Diagnosis. *Potato Research* 52(2): 173-205.
- Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. & Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science* 327(5967): 812-818.
- Gómez-Brandón, M., Lazcano, C. & Domínguez, J. (2008). The evaluation of stability and maturity during the composting of cattle manure. *Chemosphere* 70(3): 436-444.
- Goszczynska, T., Serfontein, J. & Serfontein, S. (2000). Introduction to practical phyto bacteriology. SAFRINET. *The Southern African (SADC) Loop of BioNet-International*.
- Green, S. J., Michel, F. C., Hadar, Y. & Minz, D. (2004). Similarity of bacterial communities in sawdust- and straw-amended cow manure composts. *FEMS Microbiology Letters* 233(1): 115-123.
- Hadar, Y. (2011). Suppressive compost: when plant pathology met microbial ecology. *Phytoparasitica* 39(4): 311-314.
- Hadar, Y. & Papadopoulou, K. K. (2012). Suppressive composts: microbial ecology links between abiotic environments and healthy plants. *Annual Review of Phytopathology* 50: 133-153.
- Haggag, W. & Timmusk, S. (2008). Colonization of peanut roots by biofilm - forming *Paenibacillus polymyxa* initiates biocontrol against crown rot disease. *Journal of Applied Microbiology* 104(4): 961-969.
- Haggag, W. M. & Saber, M. (2007). Suppression of early blight on tomato and purple blight on onion by foliar sprays of aerated and non-aerated compost teas. *Journal of Food Agriculture and Environment* 5(2): 302.
- Hanuš, L. O., Řezanka, T., Dembitsky, V. M. & Moussaieff, A. (2005). Myrrh-commiphora chemistry. *Biomedical papers* 149(1): 3-28.
- Hao, W., Li, H., Hu, M., Yang, L. & Rizwan-ul-Haq, M. (2011). Integrated control of citrus green and blue mold and sour rot by *Bacillus amyloliquefaciens* in combination with tea saponin. *Postharvest Biology and Technology* 59(3): 316-323.
- Hargreaves, J., Adl, M. S., Warman, P. R. & Rupasinghe, H. P. V. (2008). The effects of organic amendments on mineral element uptake and fruit quality of raspberries. *Plant and Soil* 308(1): 213-226.
- Hassan, M., Bereika, M., Abo-Elnaga, H. & Sallam, M. (2009). Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. *The Plant Pathology Journal* 25(4): 352-360.
- Hayward, A. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Review of Phytopathology* 29(1): 65-87.
- Hayward, A. (2000). *Ralstonia solanacearum*. In *Encyclopedia of microbiology*, Vol. 4 (Ed J. Lederberg). San Diego, CA: Academic Press.
- Hayward, A. C. (1964). Characteristics of *Pseudomonas solanacearum*. *Journal of Applied Bacteriology* 27(2): 265-277.
- Hirpa, A., Meuwissen, M. P. M., Tesfaye, A., Lommen, W. J. M., Oude Lansink, A., Tsegaye, A. & Struik, P. C. (2010). Analysis of Seed Potato Systems in Ethiopia. *American Journal of Potato Research* 87(6): 537-552.
- Hoffland, E., Jeger, M. J. & van Beusichem, M. L. (2000). Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. *Plant and Soil* 218(1): 239-247.
- Hoitink, H., Boehm, M. & Hadar, Y. (1993). Mechanisms of suppression of soilborne plant pathogens in compost-amended substrates. In *Science and engineering of composting: Design, environmental, microbiological and utilization aspects*, 601-621 (Ed H. A. J. H. a. H. M. Keener). Renaissance Publications, Worthington, OH.

- Hoitink, H. & Changa, C. (2002). Production and utilization guidelines for disease suppressive composts. In *XXVI International Horticultural Congress: Managing Soil-Borne Pathogens: A Sound Rhizosphere to Improve Productivity in 635*, 87-92.
- Hoitink, H., Stone, A. & Han, D. (1997). Suppression of plant diseases by composts. *HortScience* 32(2): 184-187.
- Horsfield, A., Wicks, T., Davies, K., Wilson, D. & Paton, S. (2010). Effect of fungicide use strategies on the control of early blight (*Alternaria solani*) and potato yield. *Australasian Plant Pathology* 39(4): 368-375.
- Huse, S. M., Dethlefsen, L., Huber, J. A., Welch, D. M., Relman, D. A. & Sogin, M. L. (2008). Exploring Microbial Diversity and Taxonomy Using SSU rRNA Hypervariable Tag Sequencing. *PLoS Genetics* 4(11): e1000255.
- Hynes, R. K. & Boyetchko, S. M. (2006). Research initiatives in the art and science of biopesticide formulations. *Soil Biology and Biochemistry* 38(4): 845-849.
- Irshad, G. & Naz, M. F. A. F. (2014). Important fungal diseases of potato and their management—a brief review. *MYCOPATH* 11(1).
- Ishii, K. & Takii, S. (2003). Comparison of microbial communities in four different composting processes as evaluated by denaturing gradient gel electrophoresis analysis. *Journal of Applied Microbiology* 95(1): 109-119.
- Islam, M., Mondal, C., Hossain, I. & Meah, M. (2014). Compost tea as soil drench: an alternative approach to control bacterial wilt in brinjal. *Archives of Phytopathology and Plant Protection* 47(12): 1475-1488.
- John, R. P., Tyagi, R. D., Brar, S. K., Surampalli, R. Y. & Prévost, D. (2011). Bio-encapsulation of microbial cells for targeted agricultural delivery. *Critical Reviews in Biotechnology* 31(3): 211-226.
- Junaid, J. M., Dar, N. A., Bhat, T. A., Bhat, A. H. & Bhat, M. A. (2013). Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *International Journal of Modern Plant & Animal Sciences* 1(2): 39-57.
- Kahn, R. P. (1991). Exclusion as a Plant Disease Control Strategy. *Annual Review of Phytopathology* 29(1): 219-246.
- Kassa, B. & Chindi, A. (2013). Seed Tuber Cycle and Latent Infection for the Spread of Potato Bacterial Wilt *Ralstonia solanacearum* (Smith) a Threat for Seed Production in Ethiopia. *Asian Journal of Plant Pathology* 7(2): 74-83.
- Kassa, H., Suliman, H. & Workayew, T. (2011). Evaluation of composting process and quality of compost from coffee by-products (Coffee Husk & Pulp). *Ethiopian Journal of Environmental Studies and Management* 4(4).
- Kelman, A. (1953). The bacterial wilt caused by *Pseudomonas solanacearum*. *Technical Bulletin of North Carolina Agricultural Experiment Station* (99).
- Kelman, A. (1954). The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium. *Phytopathology* 44(12): 693-695.
- Kempe, J. & Sequeira, L. (1983). Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubers with bacteria. *Plant Disease* 67(5): 499-503.
- Ketterer, N., Fisher, B. & Weltzien, H. (1992). Biological control of Botrytis cinerea on grapevine by compost extracts and their microorganisms in pure culture. In *10. International Botrytis Symposium: Recent Advances in Botrytis Research, Heraklion, Crete (Greece), 5-10 Apr 1992*: Pudoc.
- Koné, S. B., Dionne, A., Tweddell, R. J., Antoun, H. & Avis, T. J. (2010). Suppressive effect of non-aerated compost teas on foliar fungal pathogens of tomato. *Biological Control* 52(2): 167-173.
- Kuba, T., Tschöll, A., Partl, C., Meyer, K. & Insam, H. (2008). Wood ash admixture to organic wastes improves compost and its performance. *Agriculture, Ecosystems & Environment* 127(1-2): 43-49.

- Kuo, S., Ortiz-Escobar, M., Hue, N. & Hummel, R. (2004). Composting and compost utilization for agronomic and container crops. *Recent Res. Dev. Environ. Biol* 1: 451-513.
- Lamari, L. (2002). *Assess: image analysis software for plant disease quantification*. American Phytopathological Society.
- Langarica-Fuentes, A., Zafar, U., Heyworth, A., Brown, T., Fox, G. & Robson, G. D. (2014). Fungal succession in an in-vessel composting system characterized using 454 pyrosequencing. *FEMS Microbiology Ecology* 88(2): 296-308.
- Lazcano, C., Gómez-Brandón, M. & Domínguez, J. (2008). Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* 72(7): 1013-1019.
- Lee, Y.-H., Choi, C.-W., Kim, S.-H., Yun, J.-G., Chang, S.-W., Kim, Y.-S. & Hong, J.-K. (2012). Chemical pesticides and plant essential oils for disease control of tomato bacterial wilt. *The Plant Pathology Journal* 28(1): 32-39.
- Lemaga, B., Kanzikwera, R., Kakuhenzire, R., Hakiza, J. & Manzi, G. (2001). The effect of crop rotation on bacterial wilt incidence and potato tuber yield. *African Crop Science Journal* 9(1): 257-266.
- Lemenih, M. & Kassa, H. (2011). Gums and resins of Ethiopia. Center for International Forestry Research (CIFOR), Bogor, Indonesia.
- Lemenith, M. & Teketay, D. (2003). Frankincense and myrrh resources of Ethiopia: II. Medicinal and industrial uses. *SINET: Ethiopian Journal of Science* 26(2): 161-172.
- Lemessa, F. & Zeller, W. (2007). Isolation and characterisation of *Ralstonia solanacearum* strains from *Solanaceae* crops in Ethiopia. *Journal of Basic Microbiology* 47(1): 40-49.
- Letourneau, D. & van Bruggen, A. (2006). Crop protection in organic agriculture. *Organic agriculture: a global perspective*. CSIRO Publishing, Collingwood: 93-121.
- Li, J.-G. & Dong, Y.-H. (2013). Effect of a rock dust amendment on disease severity of tomato bacterial wilt. *Antonie Van Leeuwenhoek* 103(1): 11-22.
- Lima, G., Spina, A., Castoria, R., De Curtis, F. & De Cicco, V. (2005). Integration of biocontrol agents and food-grade additives for enhancing protection of stored apples from *Penicillium expansum*. *Journal of Food Protection*® 68(10): 2100-2106.
- Lindahl, B. D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjoller, R., Kõljalg, U., Pennanen, T., Rosendahl, S. & Stenlid, J. (2013). Fungal community analysis by high - throughput sequencing of amplified markers – a user's guide. *New Phytologist* 199(1): 288-299.
- Litterick, A., Harrier, L., Wallace, P., Watson, C. & Wood, M. (2004). The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—A review. *Critical Reviews in Plant Sciences* 23(6): 453-479.
- Liu, L., Sun, C., Liu, S., Chai, R., Huang, W., Liu, X., Tang, C. & Zhang, Y. (2015). Bioorganic Fertilizer Enhances Soil Suppressive Capacity against Bacterial Wilt of Tomato. *PloS one* 10(4).
- Luiz, C., Filipini, R., Costa, M. & Di Piero, R. (2012). Polysaccharides from *Aloe barbadensis* reduce the severity of bacterial spot and activate disease-related proteins in tomato. *Journal of Plant Pathology* 94(2).
- Ma, X., Wang, X., Cheng, J., Nie, X., Yu, X., Zhao, Y. & Wang, W. (2015). Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficiency against *Rhizoctonia solani* in tomato. *Biological Control* 90: 34-41.
- Mahaffee, W., Scheuerell, S., Bailey, M., Lilley, A., Timms-Wilson, T. & Spencer-Phillips, P. (2006). Compost teas: Alternative approaches to the biological control of plant diseases. *Microbial ecology of aerial plant surfaces*: 165-179.
- Maloy, O. C. (2005). Plant disease management. *The plant health instructor* 10.
- Marin, F., Santos, M., Dianez, F., Carretero, F., Gea, F. J., Yau, J. A. & Navarro, M. J. (2013). Characters of compost teas from different sources and their suppressive effect on fungal phytopathogens. *World Journal of Microbiology & Biotechnology*.

- McLaughlin, R. J., Sequeira, L. & Weingartner, D. P. (1990). Biocontrol of bacterial wilt of potato with an avirulent strain of *Pseudomonas solanacearum*: interactions with root-knot nematodes. *American Potato Journal* 67(2): 93-107.
- McQuilken, M., Whipps, J. & Lynch, J. (1994). Effects of water extracts of a composted manure-straw mixture on the plant pathogen *Botrytis cinerea*. *World Journal of Microbiology and Biotechnology* 10(1): 20-26.
- Md Islam, T. & Toyota, K. (2004). Suppression of bacterial wilt of tomato by *Ralstonia solanacearum* by incorporation of composts in soil and possible mechanisms. *Microbes and Environments* 19(1): 53-60.
- Mengesha, W. K., Powell, S. M., Evans, K. J. & Barry, K. M. (2017). Diverse microbial communities in non-aerated compost teas suppress bacterial wilt. *World Journal of Microbiology and Biotechnology* 33(3): 49.
- Messiha, N. A. S., van Diepeningen, A. D., Wenneker, M., van Beuningen, A. R., Janse, J. D., Coenen, T. G. C., Termorshuizen, A. J., van Bruggen, A. H. C. & Blok, W. J. (2007). Biological Soil Disinfestation (BSD), a new control method for potato brown rot, caused by *Ralstonia solanacearum* race 3 biovar 2. *European Journal of Plant Pathology* 117(4): 403-415.
- Misra, R., Roy, R. & Hiraoka, H. (2003). On-farm composting methods. Rome, Italy: UN-FAO.
- Moral, R., Paredes, C., Bustamante, M., Marhuenda-Egea, F. & Bernal, M. (2009). Utilisation of manure composts by high-value crops: Safety and environmental challenges. *Bioresource Technology* 100(22): 5454-5460.
- Morris, C. E., Monier, J. & Jacques, M. (1997). Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. *Applied and Environmental Microbiology* 63(4): 1570-1576.
- Mussatto, S. I., Machado, E. M., Martins, S. & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology* 4(5): 661-672.
- Muthoni, J. & Nyamongo, D. (2009). A review of constraints to ware Irish potatoes production in Kenya. *J Horticulture* 1: 98-102.
- Muthoni, J., Shimelis, H. & Melis, R. (2012). Management of Bacterial Wilt [*Ralstonia solanacearum* Yabuuchi et al., 1995] of Potatoes: Opportunity for Host Resistance in Kenya. *Journal of Agricultural Science* 4(9): 64.
- Naidu, Y., Meon, S., Kadir, J. & Siddiqui, Y. (2010). Microbial starter for the enhancement of biological activity of compost tea. *Int. J. Agric. Biol* 12(1): 51-56.
- Naidu, Y., Meon, S. & Siddiqui, Y. (2012). In vitro and in vivo evaluation of microbial-enriched compost tea on the development of powdery mildew on melon. *BioControl* 57(6): 827-836.
- Negassa, W., Abera, T., Friesen, D., Deressa, A. & Dinsa, B. (2001). Evaluation of compost for maize production under farmers' conditions. In *Seventh Eastern and Southern Africa Regional Maize Conference*, 382-386.
- Neher, D. A., Weicht, T. R., Bates, S. T., Leff, J. W. & Fierer, N. (2013). Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times.
- Nellemann, C. (2009). *The environmental food crisis: the environment's role in averting future food crises: a UNEP rapid response assessment*. UNEP/Earthprint.
- Nguyen, M. & Ranamukhaarachchi, S. (2010). Soil-borne antagonists for biological control of bacterial wilt disease caused by *Ralstonia solanacearum* in tomato and pepper. *Journal of Plant Pathology*: 395-405.
- Nigussie, A., Kuyper, T. W. & Neergaard, A. d. (2015). Agricultural waste utilisation strategies and demand for urban waste compost: Evidence from smallholder farmers in Ethiopia. *Waste Management* 44: 82-93.
- Noble, R. & Roberts, S. (2004). Eradication of plant pathogens and nematodes during composting: a review. *Plant Pathology* 53(5): 548-568.
- Oerke, E.-C. (2006). Crop losses to pests. *The Journal of Agricultural Science* 144(01): 31-43.

- Oerke, E. C. & Dehne, H. W. (2004). Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection* 23(4): 275-285.
- On, A., Wong, F., Ko, Q., Tweddell, R. J., Antoun, H. & Avis, T. J. (2015a). Antifungal effects of compost tea microorganisms on tomato pathogens. *Biological Control* 80: 63-69.
- On, A., Wong, F., Ko, Q., Tweddell, R. J., Antoun, H. & Avis, T. J. (2015b). Antifungal effects of compost tea microorganisms on tomato pathogens. *Biological Control* 80: 63-69.
- Pal, K. K. & Gardener, B. M. (2006). Biological control of plant pathogens. *The plant health instructor* 2: 1117-1142.
- Palmer, A. K., Evans, K. J. & Metcalf, D. A. (2010). Characters of aerated compost tea from immature compost that limit colonization of bean leaflets by *Botrytis cinerea*. *Journal of Applied Microbiology* 109(5): 1619-1631.
- Pane, C., Celano, G., Vilecco, D. & Zaccardelli, M. (2012). Control of *Botrytis cinerea*, *Alternaria alternata* and *Pyrenochaeta lycopersici* on tomato with whey compost-tea applications. *Crop Protection* 38: 80-86.
- Pane, C., Celano, G. & Zaccardelli, M. (2014). Metabolic patterns of bacterial communities in aerobic compost teas associated with potential biocontrol of soilborne plant diseases. *Phytopathologia Mediterranea* 53(2): 277.
- Pant, A. P., Radovich, T. J. K., Hue, N. V. & Paull, R. E. (2012). Biochemical properties of compost tea associated with compost quality and effects on pak choi growth. *Scientia Horticulturae* 148: 138-146.
- Partanen, P., Hultman, J., Paulin, L., Auvinen, P. & Romantschuk, M. (2010). Bacterial diversity at different stages of the composting process. *BMC Microbiology* 10(1): 1.
- Pleysier, C., Bayliss, K., Dell, B. & Hardy, G. S. J. (2006). Temperature, humidity, wounding and leaf age influence the development of *Alternaria alternata* lesions on leaves of *Paulownia fortunei*. *Australasian Plant Pathology* 35(3): 329-333.
- Pradhanang, P., Ji, P., Momol, M., Olson, S., Mayfield, J. & Jones, J. (2005). Application of acibenzolar-S-methyl enhances host resistance in tomato against *Ralstonia solanacearum*. *Plant Disease* 89(9): 989-993.
- Preethu, D., Bhanu Prakash, B., Srinivasamurthy, C. & Vasanthi, B. (2007). Maturity indices as an index to evaluate the quality of compost of coffee waste blended with other organic wastes. In *Proceeding of international conference on sustainable solid waste management, Chennai, India*, 270-275: Citeseer.
- Raja, A. F., Ali, F., Khan, I. A., Shawl, A. S., Arora, D. S., Shah, B. A. & Taneja, S. C. (2011). Antistaphylococcal and biofilm inhibitory activities of acetyl-11-keto- $\beta$ -boswellic acid from *Boswellia serrata*. *BMC Microbiology* 11(1): 1-9.
- Reis, R., De Goes, A., Mondal, S., Shilts, T., Brentu, F. & Timmer, L. (2006). Effect of lesion age, humidity, and fungicide application on sporulation of *Alternaria alternata*, the cause of brown spot of tangerine. *Plant Disease* 90(8): 1051-1054.
- Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K. & Swings, J. (2003a). Microbiological aspects of biowaste during composting in a monitored compost bin. *Journal of Applied Microbiology* 94(1): 127-137.
- Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H. & Swings, J. (2003b). A survey of bacteria and fungi occurring during composting and self-heating processes. *Annals of microbiology* 53(4): 349-410.
- Sackenheim, R., Weltzien, H. & Kast, W. (1994). Effects of microflora composition in the phyllosphere on biological regulation of grapevine fungal diseases. *VITIS-Journal of Grapevine Research* 33(4): 235.
- Sang, M. K. & Kim, K. D. (2011). Biocontrol Activity and Primed Systemic Resistance by Compost Water Extracts Against Anthracnoses of Pepper and Cucumber. *Phytopathology* 101(6): 732-740.
- SAS Institute, I. (2011). SAS® 9.3. Software. SAS Institute, Inc. Cary, NC.

- Scheuerell, S. & Mahaffee, W. (2002). Compost tea: principles and prospects for plant disease control. *Compost Science & Utilization* 10(4): 313-338.
- Scheuerell, S. J. (2004). Compost tea production practices, microbial properties, and plant disease suppression. In *International Conference on Soil and Compost Eco-Biology*.
- Scheuerell, S. J. & Mahaffee, W. F. (2004). Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology* 94(11): 1156-1163.
- Scheuerell, S. J. & Mahaffee, W. F. (2006). Variability Associated with Suppression of Gray Mold (*Botrytis cinerea*) on Geranium by Foliar Applications of Nonaerated and Aerated Compost Teas. *Plant Disease* 90(9): 1201-1208.
- Schisler, D., Slininger, P., Behle, R. & Jackson, M. (2004). Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology* 94(11): 1267-1271.
- Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9(7): 671-675.
- Schönfeld, J., Gelsomino, A., van Overbeek, L. S., Gorissen, A., Smalla, K. & van Elsas, J. D. (2003). Effects of compost addition and simulated solarisation on the fate of *Ralstonia solanacearum* biovar 2 and indigenous bacteria in soil. *FEMS Microbiology Ecology* 43(1): 63-74.
- Schuck, S., Weinhold, A., Luu, V. T. & Baldwin, I. T. (2014). Isolating Fungal Pathogens from a Dynamic Disease Outbreak in a Native Plant Population to Establish Plant-Pathogen Bioassays for the Ecological Model Plant *Nicotiana attenuata*. *PloS one* 9(7): e102915.
- Schulte-Geldermann, E. (2013). Tackling low potato yields in Eastern Africa: an overview of constraints and potential strategies. In *National Workshop on Seed Potato Tuber Production and Dissemination* (Ed G. Woldegiorgis, Schulz, S., Berihun, B.). Ethiopia: International Potato Center.
- Secor, G. A. & Gudmestad, N. C. (1999). Managing fungal diseases of potato. *Canadian Journal of Plant Pathology* 21(3): 213-221.
- Segarra, G., Reis, M., Casanova, E. & Trillas, M. (2009). Control of powdery mildew (*Erysiphe polygoni*) in tomato by foliar applications of compost tea. *Journal of Plant Pathology*: 683-689.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S. & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome biology* 12(6): 1.
- Sharma, R., Singh, D. & Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control* 50(3): 205-221.
- Shemekite, F., Gómez-Brandón, M., Franke-Whittle, I. H., Praehauser, B., Insam, H. & Assefa, F. (2014). Coffee husk composting: an investigation of the process using molecular and non-molecular tools. *Waste Management* 34(3): 642-652.
- Shiomi, Y., Nishiyama, M., Onizuka, T. & Marumoto, T. (1999). Comparison of bacterial community structures in the rhizoplane of tomato plants grown in soils suppressive and conducive towards bacterial wilt. *Applied and Environmental Microbiology* 65(9): 3996-4001.
- Shrestha, K., Adetutu, E. M., Shrestha, P., Walsh, K. B., Harrower, K. M., Ball, A. S. & Midmore, D. J. (2011a). Comparison of microbially enhanced compost extracts produced from composted cattle rumen content material and from commercially available inocula. *Bioresource Technology* 102(17): 7994-8002.
- Shrestha, K., Shrestha, P., Walsh, K. B., Harrower, K. M. & Midmore, D. J. (2011b). Microbial enhancement of compost extracts based on cattle rumen content compost—characterisation of a system. *Bioresource Technology* 102(17): 8027-8034.
- Siddiqui, Y., Meon, S., Ismail, M. R. & Ali, A. (2008). Trichoderma-fortified compost extracts for the control of choanephora wet rot in okra production. *Crop Protection* 27(3): 385-390.
- Siddiqui, Y., Meon, S., Ismail, R. & Rahmani, M. (2009). Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. *Biological Control* 49(1): 38-44.



- Siddiqui, Y., Naidu, Y. & Ali, A. (2015). Bio-intensive Management of Fungal Diseases of Fruits and Vegetables Utilizing Compost and Compost Teas. In *Organic Amendments and Soil Suppressiveness in Plant Disease Management*, 307-329 (Eds M.K. Meghvansi and A. Varma). Springer.
- Slavov, S., Mayama, S. & Atanassov, A. (2004). Some aspects of epidemiology of *Alternaria alternata* tobacco pathotype. *Biotechnology & Biotechnological Equipment* 18(2): 28-33.
- Soleimani, M. & Kirk, W. (2012). Enhance resistance to *Alternaria alternata* causing potato brown leaf spot disease by using some plant defense inducers. *Journal of Plant Protection Research* 52(1): 83-90.
- Song, C., Li, M., Jia, X., Wei, Z., Zhao, Y., Xi, B., Zhu, C. & Liu, D. (2014). Comparison of bacterial community structure and dynamics during the thermophilic composting of different types of solid wastes: anaerobic digestion residue, pig manure and chicken manure. *Microbial Biotechnology* 7(5): 424-433.
- Sorokulova, I., Olsen, E. & Vodyanoy, V. (2015). Biopolymers for sample collection, protection, and preservation. *Applied Microbiology and Biotechnology* 99(13): 5397-5406.
- Soumaré, M., Demeyer, A., Tack, F. & Verloo, M. (2002). Chemical characteristics of Malian and Belgian solid waste composts. *Bioresource Technology* 81(2): 97-101.
- Sparrow, L. A. & Wilson, C. R. (2012). Managing and Monitoring Viral and Soil-Borne Pathogens in Tasmanian Potato Crops. In *Sustainable Potato Production: Global Case Studies*, 309-325 (Eds Z. He, R. Larkin and W. Honeycutt). Dordrecht: Springer Netherlands.
- St Martin, C. C. (2015). Enhancing Soil Suppressiveness Using Compost and Compost Tea. In *Organic Amendments and Soil Suppressiveness in Plant Disease Management*, 25-49: Springer.
- St. Martin, C. C. G. & Brathwaite, R. A. I. (2012). Compost and compost tea: Principles and prospects as substrates and soil-borne disease management strategies in soil-less vegetable production. *Biological Agriculture & Horticulture* 28(1): 1-33.
- Strange, R. N. & Scott, P. R. (2005). Plant Disease: A Threat to Global Food Security. *Annual Review of Phytopathology* 43(1): 83-116.
- Sturz, A., Lynch, D., Martin, R. & Driscoll, A. (2006). Influence of compost tea, powdered kelp, and Manzate® 75 on bacterial-community composition, and antibiosis against *Phytophthora infestans* in the potato phylloplane. *Canadian Journal of Plant Pathology* 28(1): 52-62.
- Sudisha, J., Niranjan-Raj, S. & Shetty, H. S. (2009). Seed priming with plant gum biopolymers enhances efficacy of metalaxyl 35 SD against pearl millet downy mildew. *Phytoparasitica* 37(2): 161-169.
- Sundström, J. F., Albiñ, A., Boqvist, S., Ljungvall, K., Marstorp, H., Martiin, C., Nyberg, K., Vågsholm, I., Yuen, J. & Magnusson, U. (2014). Future threats to agricultural food production posed by environmental degradation, climate change, and animal and plant diseases – a risk analysis in three economic and climate settings. *Food Security* 6(2): 201-215.
- Tadesse, W., Desalegn, G. & Alia, R. (2007). Natural gum and resin bearing species of Ethiopia and their potential applications. *Forest Systems* 16(3): 211-221.
- Tan, S., Jiang, Y., Song, S., Huang, J., Ling, N., Xu, Y. & Shen, Q. (2013). Two *Bacillus amyloliquefaciens* strains isolated using the competitive tomato root enrichment method and their effects on suppressing *Ralstonia solanacearum* and promoting tomato plant growth. *Crop Protection* 43: 134-140.
- Tang, J.-C., Inoue, Y., Yasuta, T., Yoshida, S. & Katayama, A. (2003). Chemical and microbial properties of various compost products. *Soil Science and Plant Nutrition* 49(2): 273-280.
- Tang, J.-C., Maie, N., Tada, Y. & Katayama, A. (2006). Characterization of the maturing process of cattle manure compost. *Process Biochemistry* 41(2): 380-389.
- Teklu, E. & Hailemariam, T. (2009). Agronomic and economic efficiency of manure and urea fertilizers use on vertisols in Ethiopian highlands. *Agricultural Sciences in China* 8(3): 352-360.
- Termorshuizen, A., Van Rijn, E., Van Der Gaag, D., Alabouvette, C., Chen, Y., Lagerlöf, J., Malandrakis, A., Paplomatas, E., Rämert, B. & Ryckeboer, J. (2006). Suppressiveness of 18 composts

- against 7 pathosystems: variability in pathogen response. *Soil Biology and Biochemistry* 38(8): 2461-2477.
- Thomas, P. & Upreti, R. (2014). Testing of bacterial endophytes from non-host sources as potential antagonistic agents against tomato wilt pathogen *Ralstonia solanacearum*. *Advances in Microbiology* 2014.
- Tiquia, S. M., Wan, H. & Tam, N. F. (2002). Microbial population dynamics and enzyme activities during composting. *Compost Science & Utilization* 10(2): 150-161.
- Tusiime, G., Adipala, E., Opio, F. & Bhagsari, A. (1998). Weeds as latent hosts of *Ralstonia solanacearum* in highland Uganda: implications to development of an integrated control package for bacterial wilt. In *Bacterial Wilt Disease*, 413-419 (Ed C. A. Ph. Prio, J. Elphinstone). Berlin.: Springer.
- Tymon, L. S. (2014). Frequency of isolation, determination, and epidemiology of *Alternaria* species on potato in the Pacific Northwest. WASHINGTON STATE UNIVERSITY.
- Urban, J. & Tränkner, A. (1993). Control of grey mould (*Botrytis cinerea*) with fermented compost/water extracts. *Bulletin OILB SROP (France)*.
- Van der Waals, J., Steyn, J., Franke, A. & Haverkort, A. (2016). Grower perceptions of biotic and abiotic risks of potato production in South Africa. *Crop Protection* 84: 44-55.
- van Elsas, J. D., Chiurazzi, M., Mallon, C. A., Elhottová, D., Křišťůfek, V. & Salles, J. F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences* 109(4): 1159-1164.
- van Elsas, J. D., Kastelein, P., van Bekkum, P., van der Wolf, J. M., de Vries, P. M. & van Overbeek, L. S. (2000). Survival of *Ralstonia solanacearum* biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate climates. *Phytopathology* 90(12): 1358-1366.
- Van Reeuwijk, L. (1992). Procedures for Soil Analysis. International Soil Reference and Information Centre (ISRIC). Wageningen. Netherlands.
- Van Zwieten, M., Stovold, G. & Van Zwieten, L. (2007a). Alternatives to copper for disease control in the Australian organic industry. *A report for the Rural Industries Research and Development Corporation, RIRDC Publication* (07/110).
- Van Zwieten, M., Stovold, G. & Van Zwieten, L. (2007b). Alternatives to copper for disease control in the Australian organic industry. *A report for the Rural Industries Research and Development Corporation, RIRDC Publication* (07/110).
- Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K. & Schempp, C. M. (2007). Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine* 14(7-8): 508-516.
- Welke, S. E. (2005). The effect of compost extract on the yield of strawberries and the severity of *Botrytis cinerea*. *Journal of Sustainable Agriculture* 25(1): 57-68.
- Weller, D. M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* 26(1): 379-407.
- Weltzien, H. C. (1991). Biocontrol of foliar fungal diseases with compost extracts. In *Microbial ecology of leaves*, 430-450: Springer.
- Wickland, L., Murray, T. & Jimerson, J. (2001). Brewing up solutions to pest problems. *Biocycle* 42(3): 64-66.
- Wicks, T., Morgan, B. & Hall, B. (1996). Influence of soil fumigation and seed tuber treatment on the control of *Rhizoctonia solani* on potatoes. *Animal Production Science* 36(3): 339-345.
- Wilson, C. & Tisdell, C. (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics* 39(3): 449-462.
- Winstead, N. & Kelman, A. (1952). Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. *Phytopathology* 42(11): 628-634.
- Wu, K., Yuan, S., Wang, L., Shi, J., Zhao, J., Shen, B. & Shen, Q. (2014). Effects of bio-organic fertilizer plus soil amendment on the control of tobacco bacterial wilt and composition of soil bacterial communities. *Biology and Fertility of Soils* 50(6): 961-971.

- Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H. & Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiology and Immunology* 39(11): 897-904.
- Yao, H. & Tian, S. (2005). Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. *Journal of Applied Microbiology* 98(4): 941-950.
- Yaynu, H. (1989). Characteristics of isolates of *Pseudomonas solanacearum* in Ethiopia. *Ethiopian Journal of Agricultural Sciences* 11(1): 7-13.
- Yohalem, D., Nordheim, E. & Andrews, J. (1996). The effect of water extracts of spent mushroom compost on apple scab in the field. *Phytopathology* 86(9): 914-922.
- Yuliar, Y. A. N. & Toyota, K. (2015). Recent Trends in Control Methods for Bacterial Wilt Diseases Caused by *Ralstonia solanacearum*. *Microbes and Environments* 30(1): 1.
- Zadoks, J. C. (2003). Fifty years of crop protection, 1950–2000. *NJAS - Wageningen Journal of Life Sciences* 50(2): 181-193.
- Zaller, J. G. (2006). Foliar Spraying of Vermicompost Extracts: Effects on Fruit Quality and Indications of Late-Blight Suppression of Field-Grown Tomatoes. *Biological Agriculture & Horticulture* 24(2): 165-180.
- Zerriouh, H., de Vicente, A., Pérez-García, A. & Romero, D. (2014). Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environmental Microbiology* 16(7): 2196-2211.
- Zhan, J., Thrall, P. H. & Burdon, J. J. (2014). Achieving sustainable plant disease management through evolutionary principles. *Trends in Plant Science* 19(9): 570-575.
- Ziganshin, A. M., Liebetrau, J., Pröter, J. & Kleinstüber, S. (2013). Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials. *Applied Microbiology and Biotechnology* 97(11): 5161-5174.
- Zmora-Nahum, S., Danon, M., Hadar, Y. & Chen, Y. (2008). Chemical properties of compost extracts inhibitory to germination of *Sclerotium rolfsii*. *Soil Biology and Biochemistry* 40(10): 2523-2529.